D. BALASUBRAMANIAN

# GENES AND MEANS

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# FOREWORD

A wider definition of biotechnology includes all techniques that use living organisms or substances obtained from them to make or modify a product. This involves improvement of microbes, plants and animals. Biotechnology is essentially based on genes and their products. It aims at harnessing the genetic diversity in the living organisms for the benefit of humans. Our understanding of the genes and capabilities to manipulate them is the very basis of modern biotechnology.

The principles of genetics were formulated by an Augustinian monk — Gregor Mendel — who carried out experiments in garden pea. He showed discrete inheritance of the characters and attributed it to factors. His original paper published in 1865 was forgotten till the turn of the century. What Mendel referred as factors were redefined as genes. Elucidation of DNA structure and deciphering of the genetic code laid the foundations of molecular biology and application of this knowledge is responsible for the current interest in biotechnology. The recombinant DNA techniques have made it possible to introduce and express the gene(s) of choice from microbes, plants and animals in the desired living organisms. The genes thus furnish means for providing better products or the same products at a lower cost. Even before the dawn of recombinant DNA techniques, isolation and cloning of genes, certain genes such as those responsible for semi-dwarf rice and wheat plants contributed a great deal towards increased food production and rural prosperity in many countries including our own. Increased wheat and rice production in the country is largely due to the introduction of the two genes responsible for reduced plant height in wheat and one semi-dwarf plant type gene in rice. Though these genes have not been isolated or cloned as yet, their economic contribution is very large. At the same time defective genes are responsible for a lot of human misery and suffering expressed as genetic defects at birth are responsible for increased susceptibility to diseases at later stages of life. The long term goals are to rectify the defective genes — the so-called gene therapy.

Genetic concepts are changing rapidly and it is now realised that the genome is not as stable as was thought earlier. Besides transposable elements or jumping genes, amplification of certain

DNA sequences, gene imprinting, directed mutations, splicing and editing of the messenger RNA have been accepted as new ideas.

Classical genetics and manipulation of the genes at cellular level have played a major role in enhancing the productivity of crop plants and animals in our country. During the last ten years or so, with the efforts of the Government of India many research facilities to manipulate genes at the molecular level have been established. At the same time various programmes have been initiated to generate highly trained and skilled manpower. Molecular manipulation of genes to obtain better products requires inputs from specialists in many different areas of biology, besides specialists in other branches of science who can indigenously develop new instruments or techniques. Collaborative work towards well focussed fewer objectives is necessary for quick success in utilising modern biotechnology.

The Publications & Information Directorate[PID] is bringing out a series of popular monographs on biotechnology as a part of the Project on "Dissemination of Biotechnology Information" sponsored by the Department of Biotechnology, Government of India. The monographs would benefit school, college and university students and teachers as well as members of general public and create an awareness among them towards the newer developments in this field taking place within and outside the country and towards the enormous potential of biotechnology in improving our socioeconomic conditions. PID, which is one of the premier institutions of its type within the country, engaged in dissemination of scientific information and science popularisation for more than twentyfive years, as also the authors of the various monographs who are all highly acclaimed for their scientific contributions within the country and internationally, have joined hands in this very important venture. I am confident that the readers would find the monographs informative and enlightening and this will contribute to the development of the biotechnology within the country.

**(C.R. BHATIA)**
Secretary
Department of Biotechnology
Government of India

# PREFACE

Biotechnology is generally viewed as a brand new discipline which has been introduced about a decade or two ago. This is hardly true because it has been practised for centuries by farmers, dairymen and amateur gardeners without realising that they are practising this futuristic discipline. The major difference, however, is that they did it on the entire plant, animal or the organism in a holistic fashion whereas today the discipline has taken on a molecular dimension. It was about three generations ago that we came to realise that all the information in a biological cell is contained in the chromosomes. It came to be realised later that chromosomes are made largely of DNA molecules. Now these DNA molecules are very long, as molecules go, even as long as micrometres or millimetres. Giant chromosomes have been isolated and we are yet to know which chromosome will enter the Guinness Book of Records for being the longest. Information in these DNA molecules is apportioned in linear units called genes.

True revolution in biology has occurred in the last 50 years with the determination of the chemical structure of the gene and the understanding of how genes transfer their information and translate them in the form of proteins and how we can fish out genes from one organism and introduce them into another.

The running theme of all the seven chapters of this book is the utilisation of genes as the means for newer understanding and exploitation. As with most other exploding disciplines of science, biotechnology and genetic engineering are also full of technical words, phrases and jargon which tend to intimidate the lay reader. This is an attempt to demystify biotechnology and to share with you some of the splendours of this area. We look forward to hearing from readers and encourage you to write to us your reactions and criticism.

# ACKNOWLEDGEMENT

First of all I must acknowledge the admirable help and assistance I have received from Sukanya Datta who took the original draft and did very interesting things that have made it read a little better and also made it a little more coherent. Her enthusiasm and scholarship have been appreciable. I also thank Biman Basu for critical comments and suggestions. Bal Phondke has played no mean a role in not only cajoling me to meet the deadline but also to push me into thinking in newer directions. Without him this book would not have been a reality. All the chapters appeared earlier either in my "Speaking of Science" columns in *Newstime* and *The Hindu*. Chapter 1 appeared under the title **Playing God** in *Newstime* on 31st May 1987. Chapter 2 appeared under the title **Molecular Come Hither** signal, in *The Hindu* on 29th May 1991. Chapter 3 appeared under the title **Did Human Life Begin in Africa** in *The Hindu* on 27th March 1991. Chapter 4 appeared in a far shorter form under the title **The Genetic Factor in Diabetes** in *The Hindu* on 21st November 1990. Chapter 5 appeared in *Newstime* on 25th October 1987 under the title **The Colour Green**. Chapter 6 appeared in *The Hindu* as **Roots of Revolution** on 27th November 1991. The final chapter appeared as **The Culture in Agriculture** *in The Hindu* on 21st July 1991. I am grateful to the Editors of *Newstime* and *The Hindu* for permission to use and to modify these articles for this book.

Illustrations have been handled by the very able artist Mr Pradip Banerjee of PID. He was always willing to hear criticism and suggestions and turn them around to produce improved and novel versions. We hope that the illustrations not only enrich but also occasionally complement the text.

*Dedicated to*
*SHAKALA*

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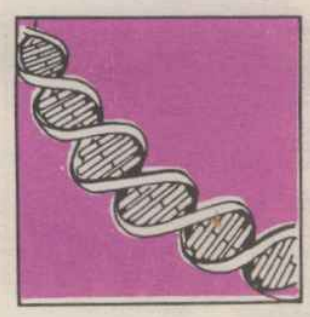
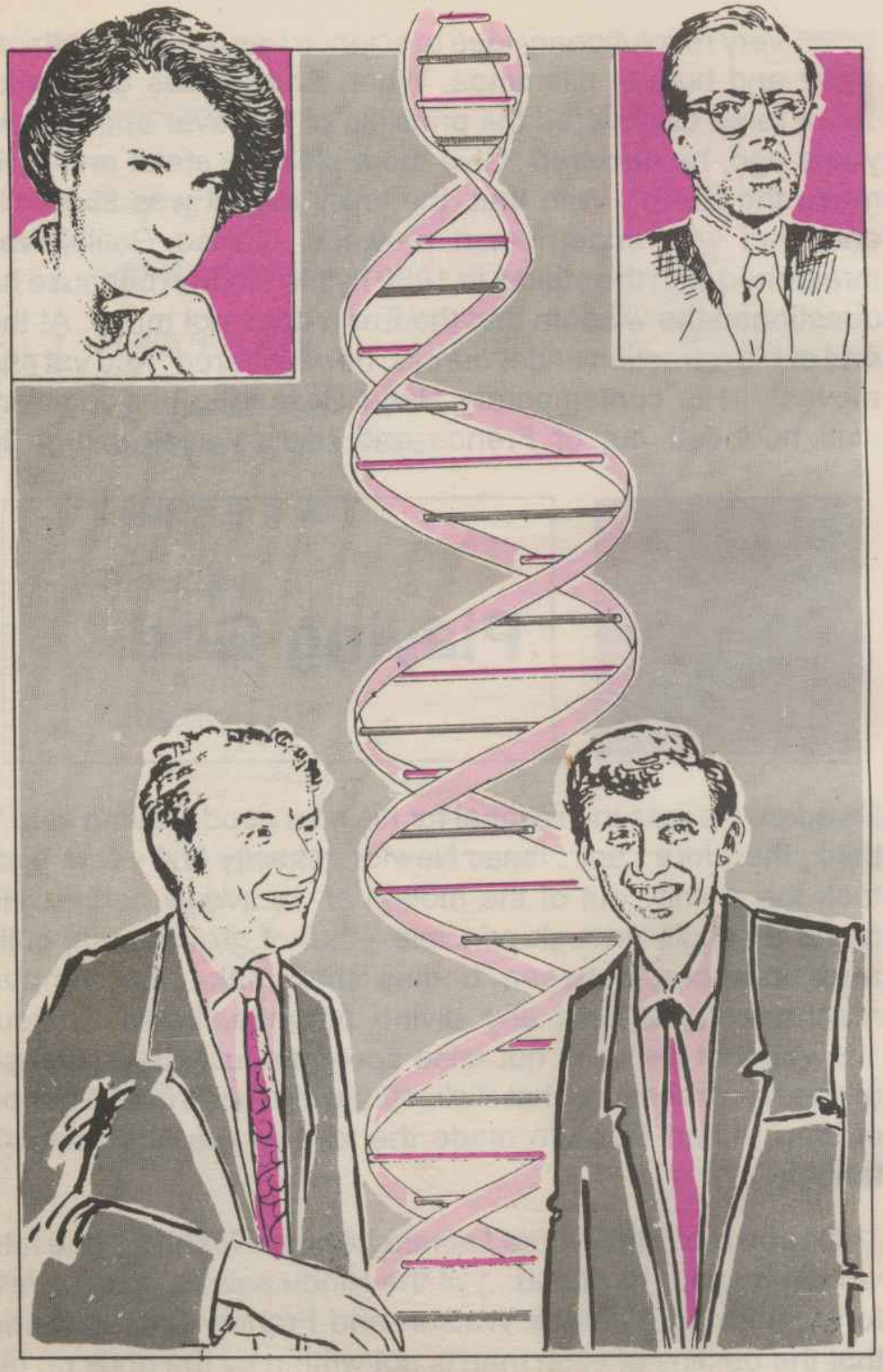
Every revolutionary idea in science has provoked ethical and human dilemmas. When Archimedes of Eureka fame discovered the principle of the lever about 2200 years ago, he declared "Give me a place to stand and I will move the Earth". With that, the lowly mortal was suddenly endowed with superhuman prowess. Galileo Galilei was threatened with the stakes in 1639 by the Church because he questioned the wisdom that the Earth does not move. At the end of his retraction under duress, he whispered "And yet she moves". His contemporary Rene Descartes, the logician, was hounded out of France and had to seek refuge in



# Playing God

Sweden. He was too rational for his own good, having said "I think, therefore I am". Isaac Newton, exactly 300 years ago, took the divinity out of the motion of heavenly bodies. He found a simple enough principle—that of gravitational pulls and balances between bodies that make them move mechanically without any divine purpose. Louis Pasteur showed that life does not arise spontaneously but because of chemical reactions that click, during evolution over a period of time. Albert Einstein made the idea of the absolute untenable.

Do you recall the words *Lalaata Likhitaa Rekhaa*... (the fate written on your forehead...) of the Hindu sacred texts? Well, forty years ago, James Watson and Francis Crick showed that the destiny of each man is not written by the gods on his forehead, but written up chemically in the **genes** of his body in a string of three-letter words using the **sequence of bases** in the DNA molecule.

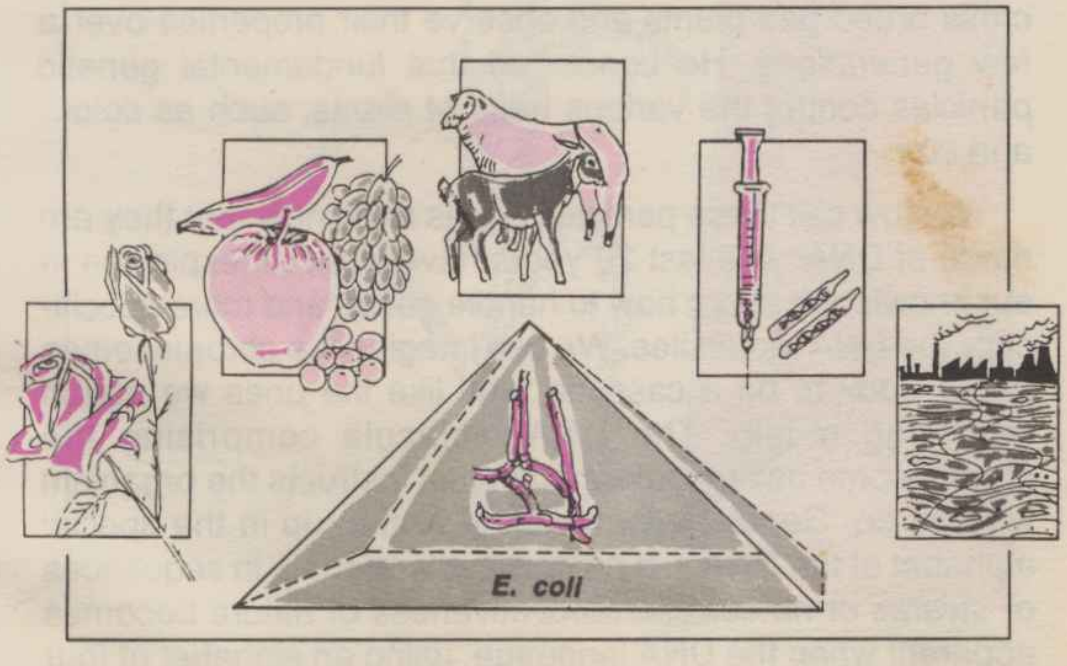


Francis Crick (left) James Watson (right),
Rosalind Franklin (top; left) and Maurice Wilkins (top; right)
unravelled the mystery of the DNA structure

To know is to use, to apply and to harness. With an increasing understanding of molecular biology new technology became possible. With the work of Watson and Crick not only did molecular biology emerge but also its applications. These came to be loosely known as genetic engineering or biotechnology.

Biotechnology is based on the knowledge of life processes which is harnessed for human benefit. Initially employed to mass produce useful substances, it did not remain confined to this task. So malleable is this new technology and so rewarding the efforts that the past few years have witnessed an explosive growth. It is now no longer a futuristic science. Instead, it touches our lives everyday in ever increasing ways.

The splendours of biotechnology are so many that it almost seems to be the science of wish-fulfillment. Bigger fruits. Brighter flowers. Higher yields. Super cattle. Exotic colours and flavours. Cheaper medicines. More efficient vaccines. The list seems endless and the cornucopia of biotechnology shows no sign of exhaustion. So much has biotechnology




Splendours of Biotechnology

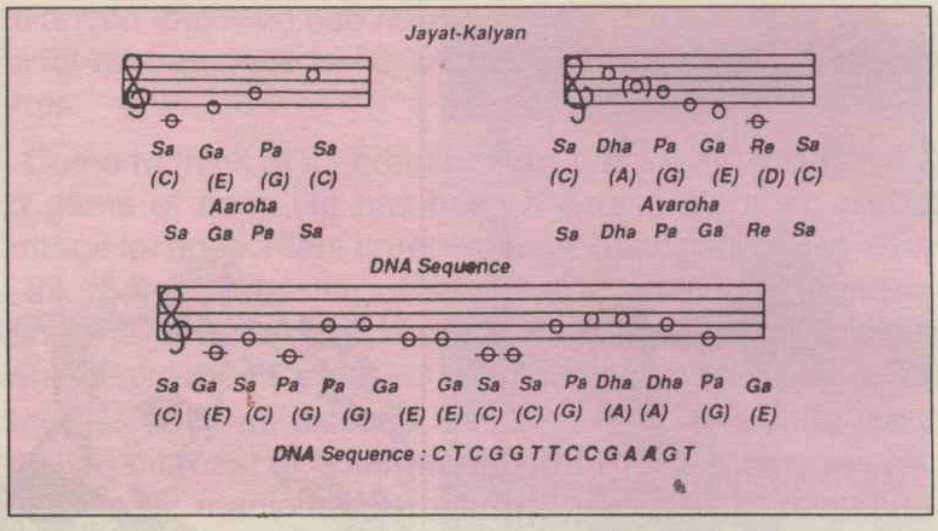
advanced that man can now play God. He can alter existing plants and animals to his own design and 'create' new life forms.

Come to think of it - creating new organisms has been an old game of man. He has been breeding plants to his advantage for ages. Plant breeders have been particularly adept at this game. Crossing of strains and grafting of flowering plants have been well known for decades. High yielding plants have been crossed with those that had other advantages such as disease resistance to give hybrid plants that had the best of both parents. Traditional crossbreeding was genetic manipulation over time because it resulted in progenies derived from two superior parental types. But it did not involve direct interference with the DNA contained in the cells. Now the modern era of biotechnology has changed all that.

The stage was set in 1856 when the father of genetics, the Austrian monk Gregor Mendel, (1822-1884) set forth the rationale of the field of biotechnology. What he did was to cross breed pea plants and observe their properties over a few generations. He concluded that fundamental genetic particles control the various traits of plants, such as colour and size.

We now call these particles genes and know that they are made of DNA. The last 20 years have seen an explosion in our knowledge about how to handle genes and more specifically the DNA molecules. We can imagine the chromosomes in our body to be a cassette tape like the ones we use in recording music. The DNA molecule comprising the chromosome has the information that instructs the organism what to do. Genetic information is written up in the special alphabet of the DNA just as music is written up in sequences of *swaras* or notes. The innovativeness of nature becomes apparent when the DNA language, using an alphabet of four bases, rivals music using seven *swaras* or musical notes, both in beauty and complexity. During reproduction and cell

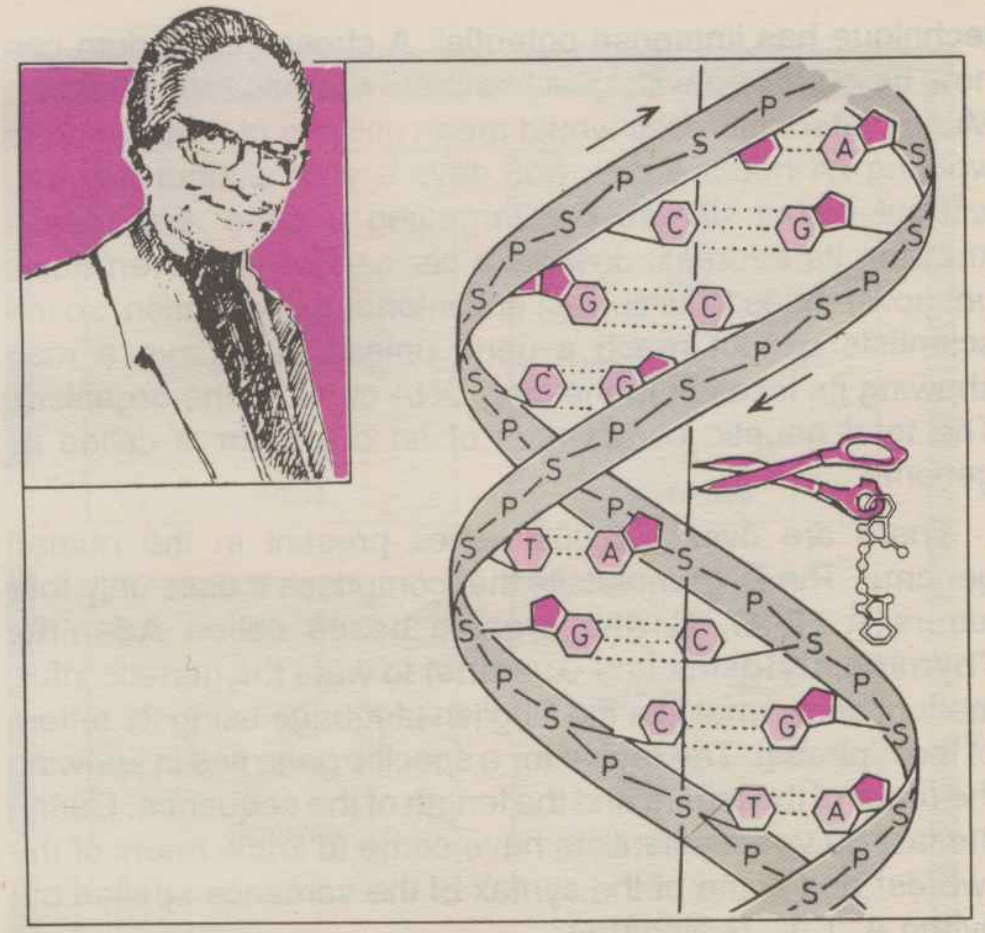
Gregor Mendel, the Father of Genetics and the abbey where he carried out his work (inset)


Jayat-Kalyan
Sa Ga Pa Sa
(C) (E) (G) (C)
Aaroha
Sa Ga Pa Sa
Sa Dha Pa Ga Re Sa
(C) (A) (G) (E) (D) (C)
Avaroha
Sa Dha Pa Ga Re Sa
DNA Sequence
Sa Ga Sa Pa Pa Ga Ga Sa Sa Pa Dha Dha Pa Ga
(C) (E) (C) (G) (G) (E) (E) (C) (C) (G) (A) (A) (G) (E)
DNA Sequence : C T C G G T T C C G A A G T

DNA music

division, the parent DNA molecules are copied into the next generation cassette with high fidelity. The offspring inherits the DNA, the genes and the chromosomes of the parents. Suppose we want to change it, so that the offspring may have a different set of DNA sequences. The way to do it is to change the sequences in the parents and transmit those with fidelity to the child. The change might be the removal of one part of the 'song', or the addition of a new song or a phrase in the copy tape. Now, when the content of the original tape is compared with, that of the transformed one and that of subsequent copies, the changes are clear. The latter two would now have the changed version and not the original information, or the trait, message of their grandparent.

With audio or video cassettes this is easy since editing machines are available. But how do we do this to the DNA at the molecular level? This is where the discovery of special enzymes comes in handy. These enzymes discovered about 20 years ago specifically hydrolyze, that is cut, the DNA molecule or the genes at chosen points. These scissoring enzymes are called restriction nucleases. One can then transfer the cut DNA segment into a different "host" cell. The




Walter Gilbert (inset): pioneer in the field of molecular tailoring

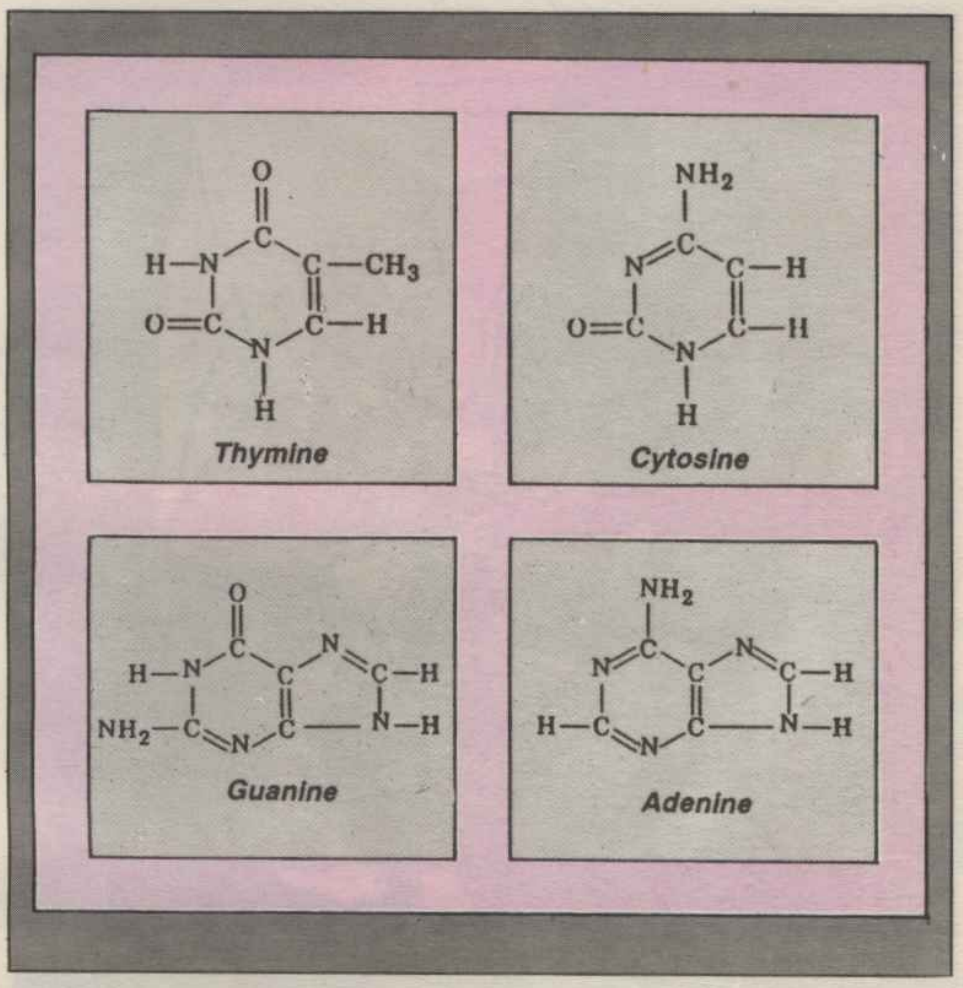
"guest" or foreign DNA can be attached, to the host cell's DNA at chosen points. Other enzymes help in this "stitching" or ligation. Hence the name ligase for these enzymes. This is true molecular tailoring! Earlier, this used to be done in a trial and error approach. With the discovery of **sequence-specific enzymes** nucleases and ligases—, molecular scissors and sewing machines—and the understanding of how to choose specific regions in the DNA to edit, this is now done with great precision. This is called gene cloning, recombinant DNA technology or genetic engineering. If the DNA of the engineered cell is not tampered with, it will also reproduce the newly added information for ever with great fidelity. We can thus obtain generations of identical cells, or a clone of cells all with identical genetic information and properties. The

technique has immense potential. A chosen bacterium can now be endowed with qualities it did not naturally possess. Manipulated microbes would mean millions of mute workers working 24 hours a day, 365 days a year without pay and without labour unrest. But targetting a gene also means knowing its address. Just as a car cannot be driven down unknown roads to its correct and intended destination, so too scientists cannot reach a gene unless they have a map showing its location in the long DNA chain of the organism. The total genetic endowment of an organism is called its genome.

There are over 100,000 genes present in the human genome. The DNA molecule that comprises it uses only four letters A, T, C, G (the organic bases called **Adenine**, **Thymine**, **Cytosine** and **Guanine**) to write the genetic information; somewhat like the English language using 27 letters of the alphabet. The recipe for a specific gene lies in knowing the order of the letters and the length of the sequence. During the last 30 years scientists have come to know many of the "words" and some of the syntax of the sentence spelled out by the A, T, C, G alphabet.

With diseases, scientists adopt a backdoor technique. They identify a disease, determine if it is inherited and work backwards till they find the gene responsible for the disease. At present without a disease or an externally visible or otherwise measurable symptom scientists do not have a clue to what the gene does. They may read a chromosome from start to finish and note down every 'letter' but the meaning of the sequence would still elude them. It is to understand the riddle of life that an ambitious project has been launched in January 1989. It is a project that rivals in scope the Manhattan Project or the Apollo moon landings. Its aim is to map the entire human genome and spell out the entire message hidden in each one of the chromosomes there.

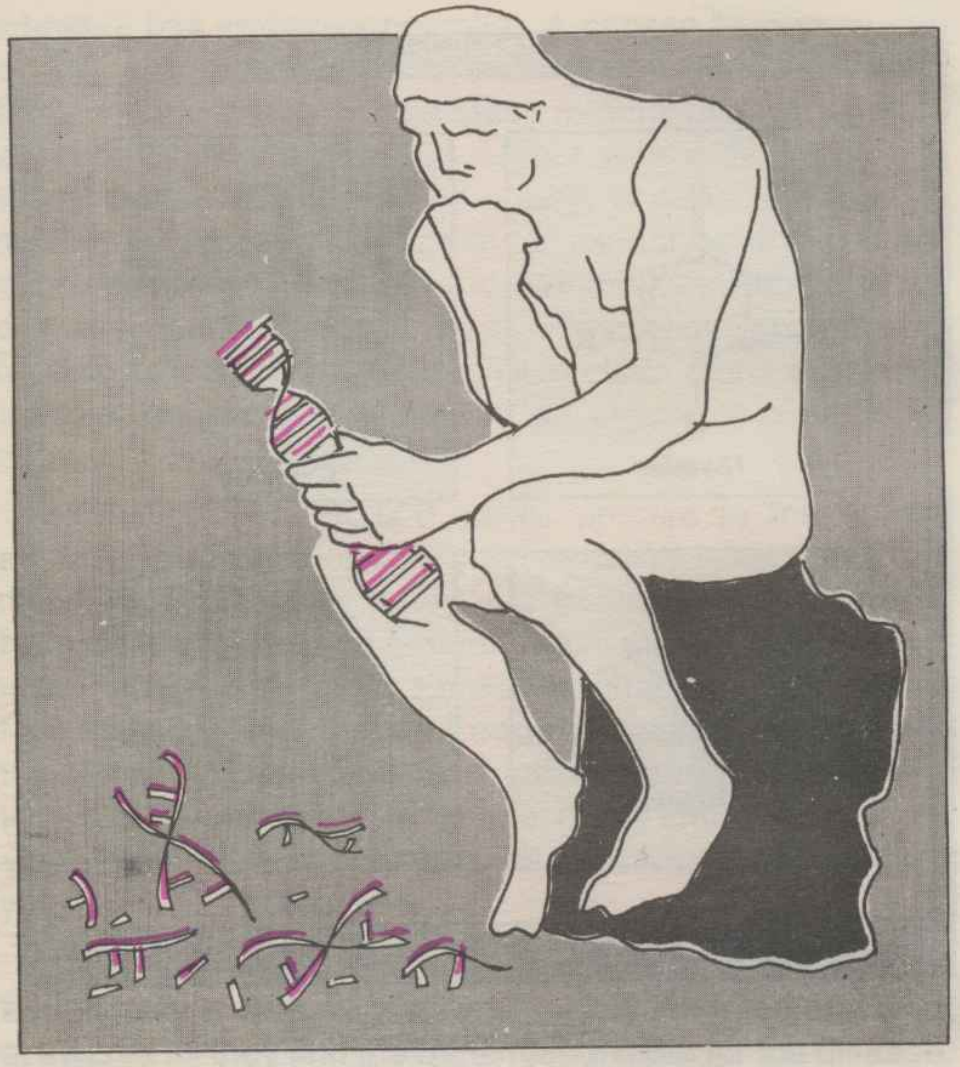
DNA is found in the human-cell nucleus in the form of 46 separate threads, each coiled to form a chromosome. Un-




DNA alphabet

ravelled and joined end to end, the DNA molecule would form a fragile thread, more than 5 feet long but only 50 billionth of an inch across, truly a *macro* molecule. In a human being the genetic message is some 3 billion letters long, contained in tens of thousands of genes strung together to make the genome. Of these about 5,000 genes have been identified, while about 1,500 have been roughly located or mapped on the various chromosomes. The Human Genome Project is clearly a long term plan and Alexander Pope's lines "know then thyself ..... the riddle of the world" appear prophetic.

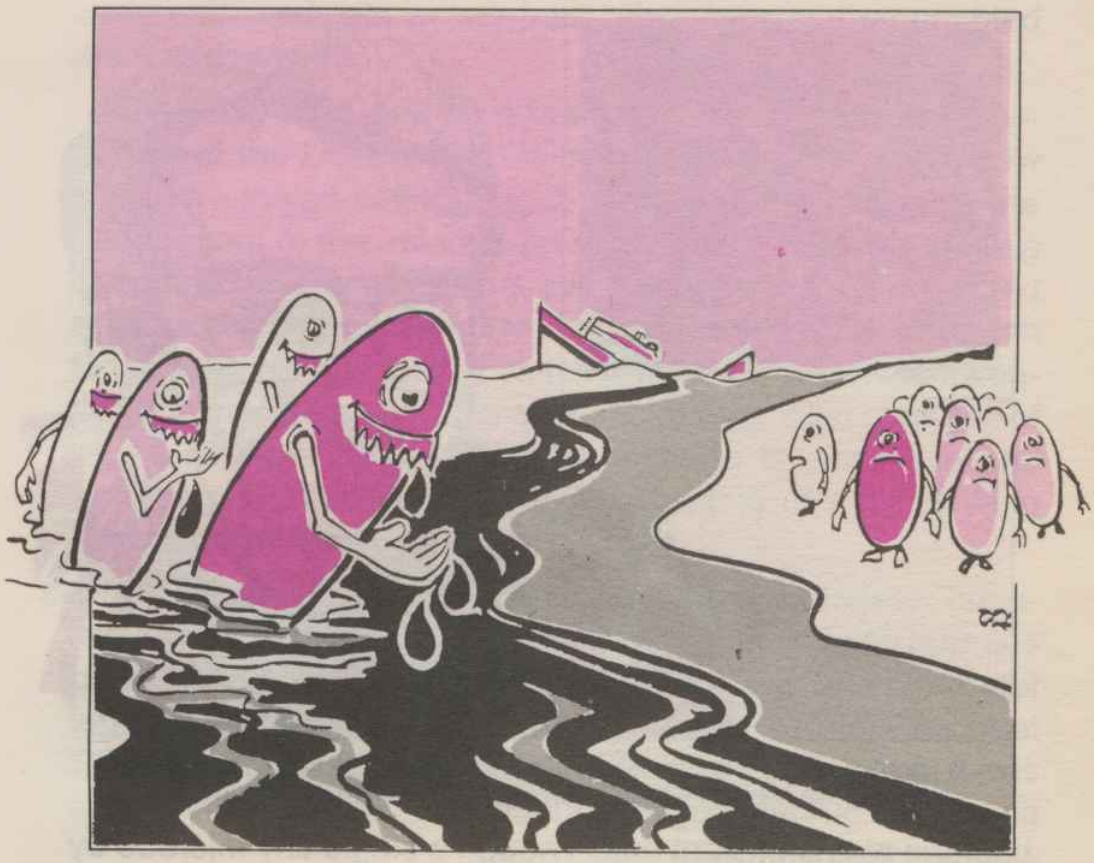
However, while the human genome project plods on scientists have not neglected the lowly bacteria either. And many



Know thyself!

of the first successes of genetic engineering have been reported on microbes.

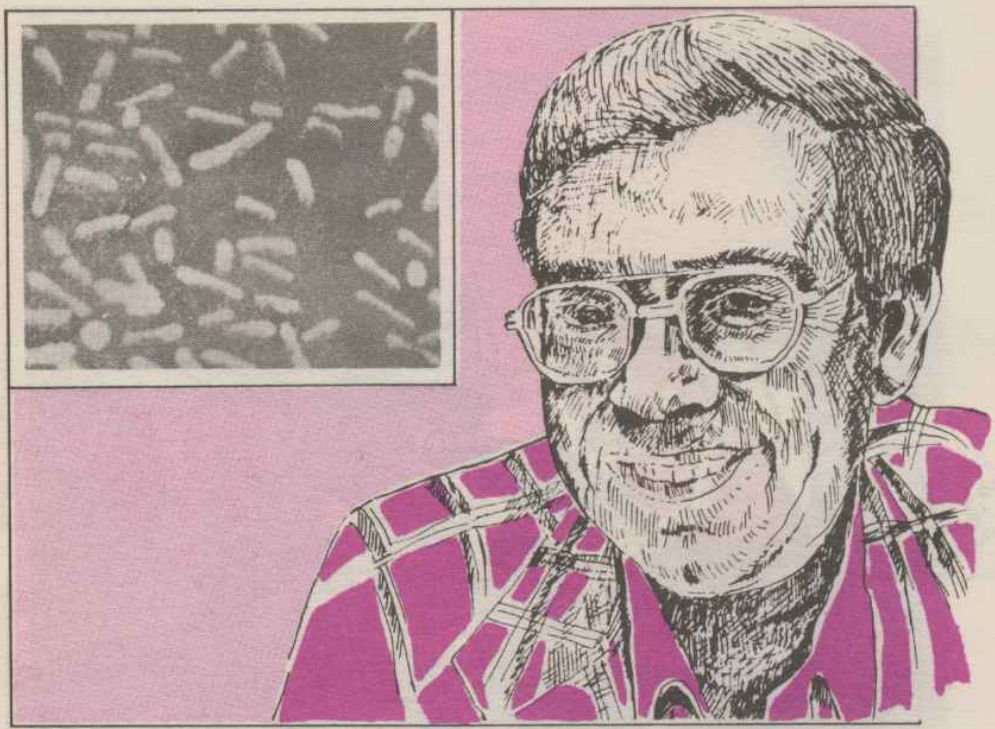
We know of bacteria in the soil that digest animal wastes or even buried carcasses. Suppose we want a bacterium which would digest petrol or crude oil instead. This would be handy for clean-up operations when oil tankers spill substantial quantities of crude oil in the sea. Just releasing these bugs at the spills would help mop-up operations. They could eat off the oil and multiply profusely to do more of the same. Before long, the area would be cleaned up. This method would be



Oil Ahoy!

much cheaper and far more convenient than the conventional alternatives. It would also be an ecologically friendly process. This is no doubt a wonderful idea except that such crude oil digesting bacteria are not available naturally.

An Indian-born American microbiologist, Ananda Mohan Chakrabarty, was intrigued by the problem. He also had a novel idea. Working then at the General Electric Company's research laboratories, he experimented with gene **mutations** in bacteria. In particular he focussed on those mutations that would endow 'wild' type bacteria with the genetic make-up that would enable them to digest and thus clean up spilled oil. Patient work for months finally yielded results. He successfully isolated, cultured and grew a population of novel bacteria that would consume and digest crude oil. The idea of using



Ananda Mohan Chakrabarty with 'superbugs' (inset)

bacteria was a brilliant one. Though a single tiny microbe by itself has no great appetite to speak of, its most important attribute is its very short generation time. It needs very little time to reproduce itself. A bacterium reproduces to yield an offspring in about half an hour. That means starting with one bacterium we end up with a million of them in just ten short hours. If they breed true, with no genetic error, the million bacteria are a true population of clones each doing its bit in cleaning up its bellyful of oilfeed. A culture of oil-eating bacteria let loose into a marine oil spill, will gobble it up within a few days and the place will be cleaned without much manual labour.

Chakrabarty's bugs too did the job for which they had been designed. Armed with this initial success on the mutant isolate, Chakraborty genetically engineered bacteria and soon had a set of clones on his hand. These were tailor-made for the job of cleaning up oil spills because they were artifi-

cially endowed with genes from other species that enabled them to digest oil.

The story now took on a dramatic turn. Chakrabarty approached the US Patents Office to file a patent on his new creation—the oil-eating bug. A patent is the granting by the government to the inventor an exclusive privilege of making or selling his new invention. His invention is thus protected and the inventor gains money by selling it to others who wish to utilise it. No problem of ethics is involved when the invention is a machine or a process to make drugs. No question of ethics is raised when the item patented is inanimate and nonliving. But the invention and patenting of a living thing raises thorny questions.

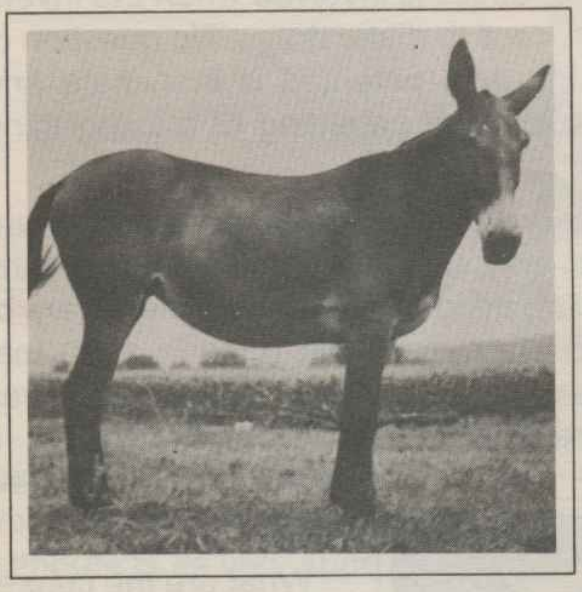
The bacterium, to be sure, is a spineless, boneless, brainless creature that has no consciousness, at least as humans define it—, but it is a living thing nonetheless. After years of legal wrangling, the US Supreme Court ruled in 1980 that Chakrabarty could obtain a patent on his oil-eating bug. It was an earth shattering, precedent creating decision. With it dawned the Brave New World.

The Geep

What are the consequences of this patent approval? The pace with which genetic engineering has been progressing in the last few years has seen the methods and organisms being experimented with become more sophisticated. Bizarre creations have already been made by biologists. Fusions of rats with mice and with

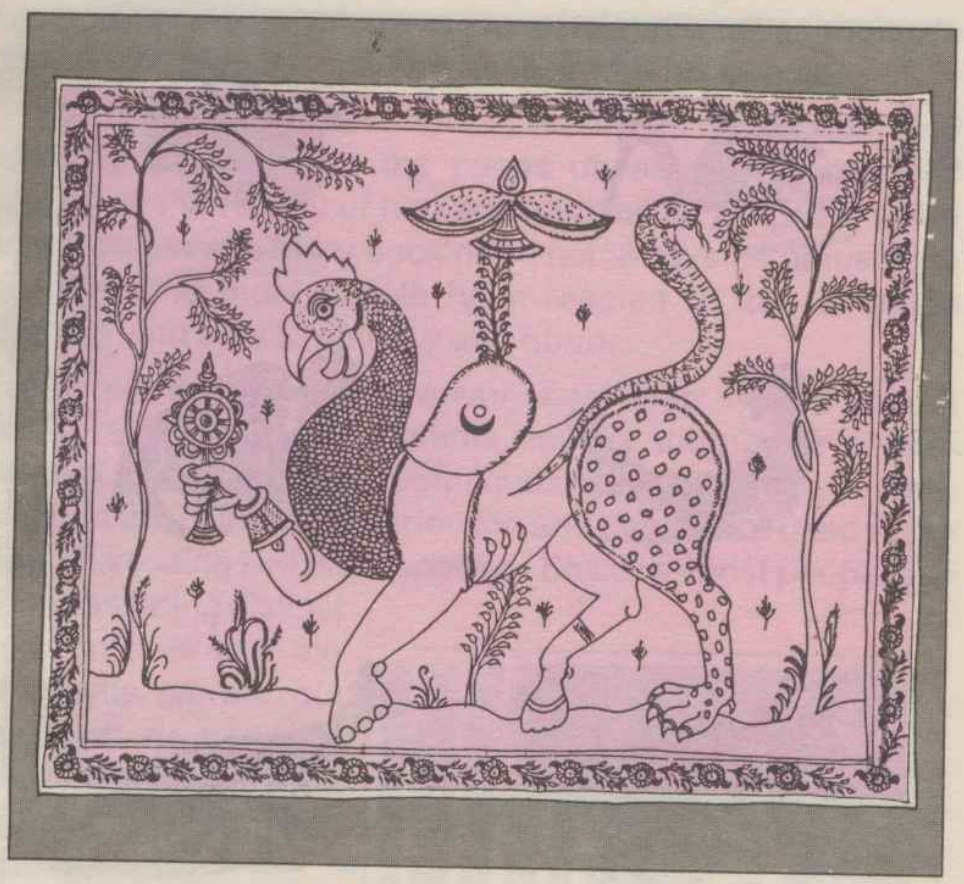
frogs have been attempted. The most disturbing creature created in a laboratory is what is called the geep. It is a composite creature, halfway between a goat and a sheep. The geep has the head and the long neck of the goat and the woolly coat, and knobby knees of the sheep.

A hybrid is the offspring of two animals or plants of different species. Usually hybrid animals are sterile but very hardy. A mule is a hybrid that is part horse and part donkey and they have been specially bred to help man to carry heavy loads specially on mountainous terrains. The geep is also a sterile genetic dead-end like the mule. But the chasm between a mule and the geep is wide. A mule is born out of a sexual cross between a pair of parent animals. The geep is a laboratory creation in the test tube and would perhaps never have seen the light of day but for the whims of man. It was made in the biology laboratory at the University of California by fusing a sheep and a goat embryo and implanting the resultant cell in a goat's womb. The fertilized cell developed in the womb of its surrogate mother. The geep that was born in 1985 is alive and literally kicking. But of what use is it? Perhaps none. Except to serve as a living proof that such embryo fusions to produce hybrid species is a practical proposition and that scientists hold the power in their hands. It is an awesome thought.



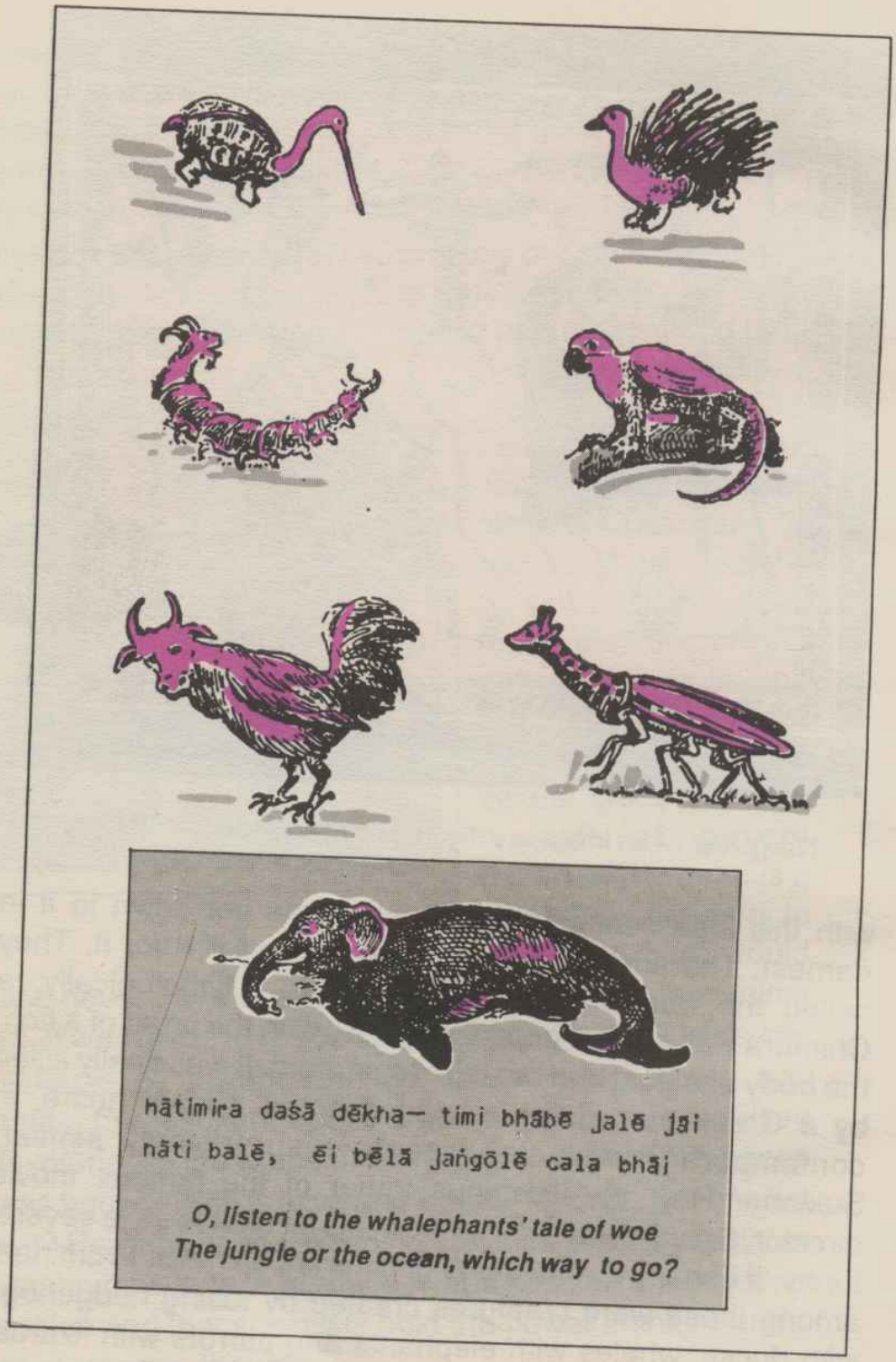
The Mule

The idea of combining two or more different species to obtain bizarre offsprings is nothing new. Mankind had toyed



Navgunjar — an imaginary figure of nine animals in one form, popular in folktales from Orissa

with this idea centuries before scientists got down to it in earnest. The ancient Greeks even had a word for it. They called the freakish monsters Chimera. Mythologically, a Chimera was a fire breathing monster with the head of a lion, the body of a goat and the tail of a serpent. It was finally killed by a Greek hero riding a winged horse. Closer home, in contemporary India, the scene is surprisingly similar. Sukumar Ray, the illustrious father of the famous movie director, Satyajit Ray, described in his inimitable style several funny, if unhappy, amalgams of different animals. Prominent among these were creatures created by fusing hedgehogs with ducks, whales with elephants and parrots with lizards.


hātimira daśā dēkha— timi bhābē jalē jāi
nāti balē, ēi bēlā jaṅgōlē cala bhāi
O, listen to the whalephants' tale of woe
The jungle or the ocean, which way to go?

The fanciful world of Sukumar Ray

The gastronomic problems of these half-and-half animals are recited with glee by children familiar with his poems.

The unicorns, griffins, hydras, centaurs, mermaids, and basilisks that populate the pages of fairy tales are proof enough of the fertility of human imagination. But what is fun on the pages of a story book need not always be amusing in real life. And most people have reacted to the lab-made chimera with shock, outrage and horror.

The plant kingdom, however, has been spared violent public rejection. Rather, plants had for long been experimented with. Grafting techniques have over the years, reached the pinnacle of perfection and people are used to the idea of crossing two plant species. Besides, most people see plants as being distinct from animals and experiments carried out on plants do not elicit the overt outbursts that animal experiments cause.

People did feel somewhat uneasy with the pomato— a hybrid plant between the potato and the tomato, or with the nectarine, the orange-tangerine combination. These have, however, been accepted in time. So have been some of the practices of animal husbandry: for example, manipulation of light in chicken coops so that hens laid more

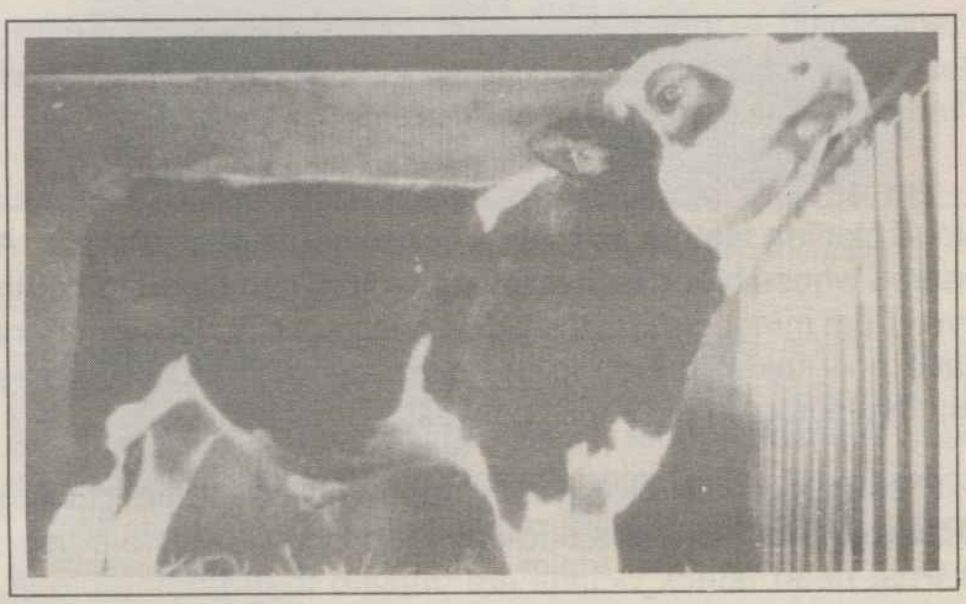
Bigger and better radishes

eggs or injecting **hormones** into cattle to help develop more muscle and more meat. But now with the creation of chimeric animals long standing moral and ethical beliefs are shaken! Added on to this has been the ruling of the US Patent Commissioner, that non-naturally occurring, non-human, multicellular organisms, including animals are patentable.

Interest groups such as the Humane Society, the American Society for the Prevention of Cruelty to Animals (ASPCA), the US Foundation on Economic Trends and religious groups were gravely concerned about the ethical and moral issues raised by genetic engineering of organisms. Two poignant questions that have been raised are - "How can the patent office encourage scientists to play God?", and "Who are these people to redefine life as being no different than a toaster or a tennis ball? How can one draw a line at the human level?"

The counter argument is worth mentioning as well. The claim is that genetically engineered animals play a guiding role in combating disease. Experimentation with them helps in the development of potential new therapy. These animals would also allow a reduction in the amount of animal testing and the extent of their suffering. A mouse model for cancer has been developed at Harvard University. In the 1980s scientists Philip Leder and Timothy Stewart discovered that they could introduce a special cancer-related gene called oncogene into the chromosome of a mouse embryo at very early stages and nurture it to produce a viable adult. The expression of the oncogene would result in abnormal cell development leading to cancer. The so engineered 'oncomouse', as it was called, soon became the centre of a controversy when the American patents office granted a patent on it in 1984. Biotechnology companies also cite the possibility of making a mouse susceptible to the human AIDS virus. Biotechnology companies are also actively considering the feasibility of creating animals with selected extra foreign genes in their cells. These animals, termed transgenic, could well be the food production factories of the future. Transgenic

cows, sheep and goats could be created which would produce special proteins in their blood or milk. By choosing the gene responsible for the expression of the protein of choice custom designed animals could be created in the laboratories.



Herman, the first transgenic bull

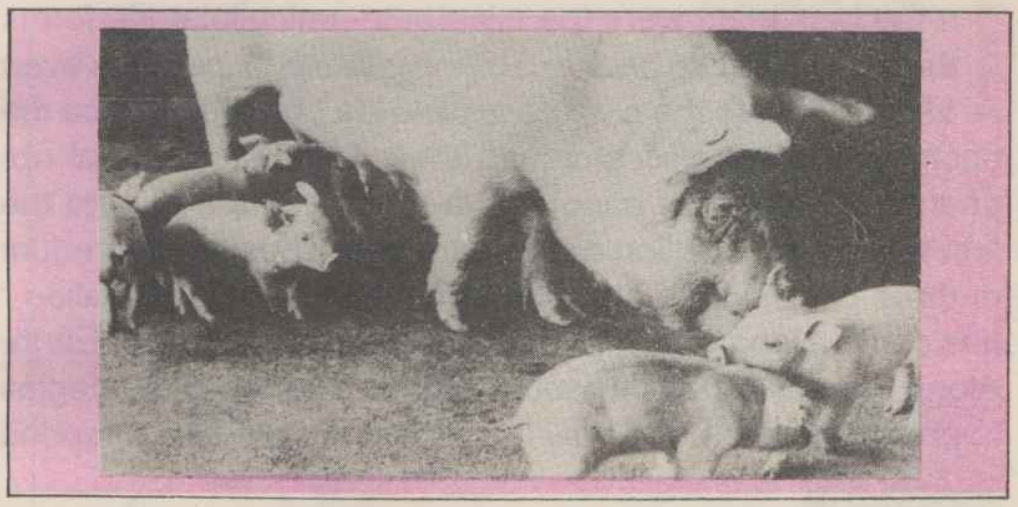
Herman and Tracy are two such animals. Herman, the bull, was 'created' by the biotechnology company Gene Pharming. This European company had wanted to implant into cows a modified version of the human gene for the protein lactoferrin. This protein is secreted in milk as part of the natural defence against infection. Gene Pharming had hoped that cows with an extra lactoferrin gene would be less susceptible to mastitis, the infection that causes an inflammation of the udder. The embryo with its newly implanted gene, however, turned out to be Herman. Gene Pharming nurtured it to adulthood in the fond hope that Herman would pass on copies of the foreign lactoferrin gene to his daughters, thus conferring on them resistance to mastitis. The company also hopes that milk from Herman's daughters could be useful as a source of human

lactoferrin to fight gut infections and diarrhoea common in AIDS patients.

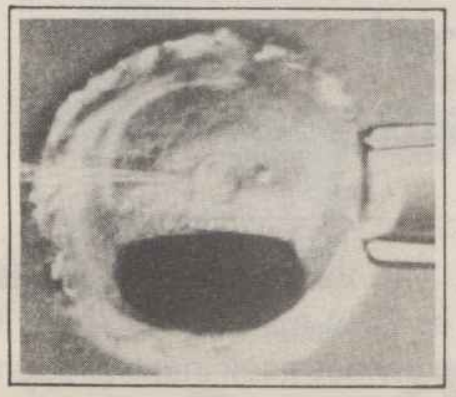
Meanwhile Pharmaceutical Proteins and the Agricultural and Food Research Council's Institute of Animal Physiology and Genetics Research, both at Edinburgh created Tracy, a transgenic sheep. Tracy secretes a human protein called $\alpha_1$ - antitrypsin in her milk. Some people have an inherited deficiency of the protein and $\alpha_1$-antitrypsin purified from Tracy's milk is expected to help them.

Yet another biotechnological aim is to produce human blood clotting factors as natural constituents of cow's milk. A person whose blood lacks clotting factors can bleed to death even from minor cuts as the blood does not clot. Researchers have now taken a step in the direction of producing these clotting factors in pig's milk as well.

Presently, most biologicals approved as blood clotting factors for hemophiliacs and anti-blood clotting factors used to treat heart attack victims are derived from human blood. But ample supply of human blood is a problem, also the blood needs to be safe and free from contamination by disease causing bacteria or pathogens. Genetically engineered farm animals could provide an alternative and possibly safer source of these medicines. Other genes, equally attractive are to be engineered into cattle. For example, cow's milk could be altered to contain more of certain proteins, such as the cheese forming casein. A 20- percent increase in the right type of casein would be worth many millions annually to cheese producers. Then again, many of the world's adults have difficulty digesting the milk sugar lactose. Reduction of this component in milk would increase the market for milk and milk products. A low-lactose milk would also benefit premium ice cream manufacturers; lactose forms unwanted crystals in their frozen products. But perhaps the ultimate animal bioreactor would be the dairy cow which can produce more than 10,000 litres of milk annually.

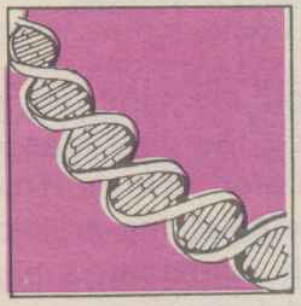


Genie with her piglets created by microinjection of foreign DNA into ova (below)



ASPCA, on the other hand, has countered the very idea of genetically altered animals. It has already objected to the practice of genetically engineered pigs that might fetch more meat and hence more dollars. ASPCA has pointed out that gene- doctored pigs have become arthritic, infection-prone and cross- eyed. So where exactly does the profit motive stop and cruelty to the animal get prevented? Clearly a public debate of the larger issues involved in these practices is warranted before patenting is allowed. But whether every country will listen to the appeal of ethics is the question. For with the powers that a biotechnological process offers also comes professional and societal responsibility. It is a double edged weapon that bestows plenty as well as holds the potential for transcending all boundaries traditionally respected by man.

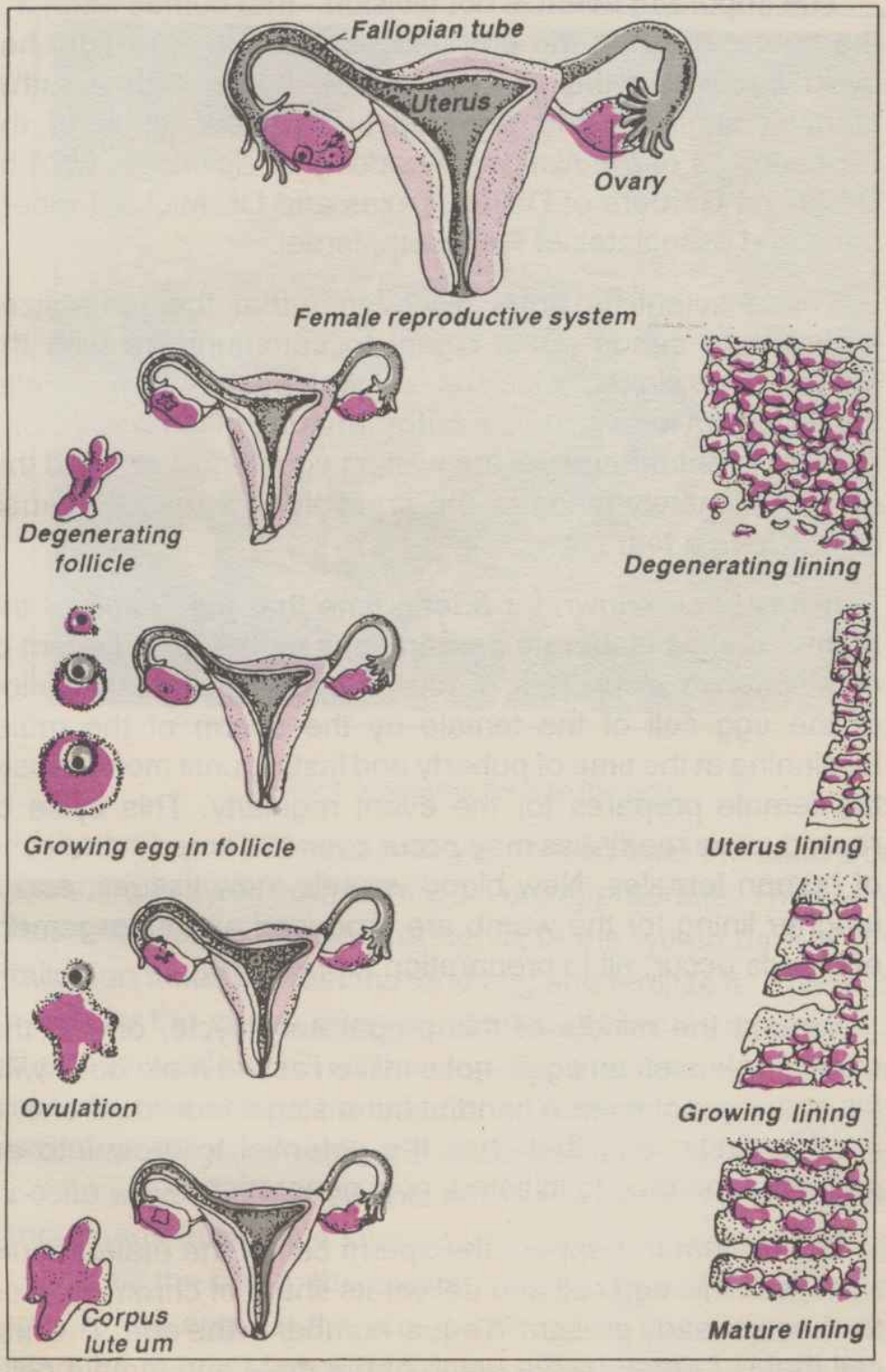
In the beginning were the microbes - unicellular life forms that can be traced back to 3 billion years ago, perhaps even earlier. Prior to them, there was no life on earth. Hence the bacterium is our ancestor. But then, the first mammal appeared on earth just about half-a-billion years ago, and the first humanoid a million or so years ago. Thus the bacterium of three billion years ago - the archaebacter as it is called - has come a long way in its evolutionary path on planet Earth. Along the way, it has devised and used a variety of strategies to propagate itself. The way many bacteria produce offspring



## Signal advances

or reproduce, is rather prosaic. The information for all its biological activity is contained in its DNA molecules. Thus, it first makes a copy of its DNA, then divides itself into two such that the original DNA is in one cell and the copy DNA in the other. The "daughter" cell is identical to the "parent". This simple mode of reproduction provides clones.

Variety is the spice of life, in more ways than one. Cloning does not produce variety, it is a strict copy making. If you need to evolve, you need to introduce variation. Here is where a remarkable "invention" occurred in nature - that of the evolution of sex. It is sex which introduces variability. The father brings his DNA in the sperm. The mother brings hers in the ovum. The sperm and the ovum should meet and fuse. Only then can reproduction start. This mix and match strategy brings a larger choice of DNA - genes and chromosomes - for manifesting better traits to cope with the world.




The womb makes elaborate preparations

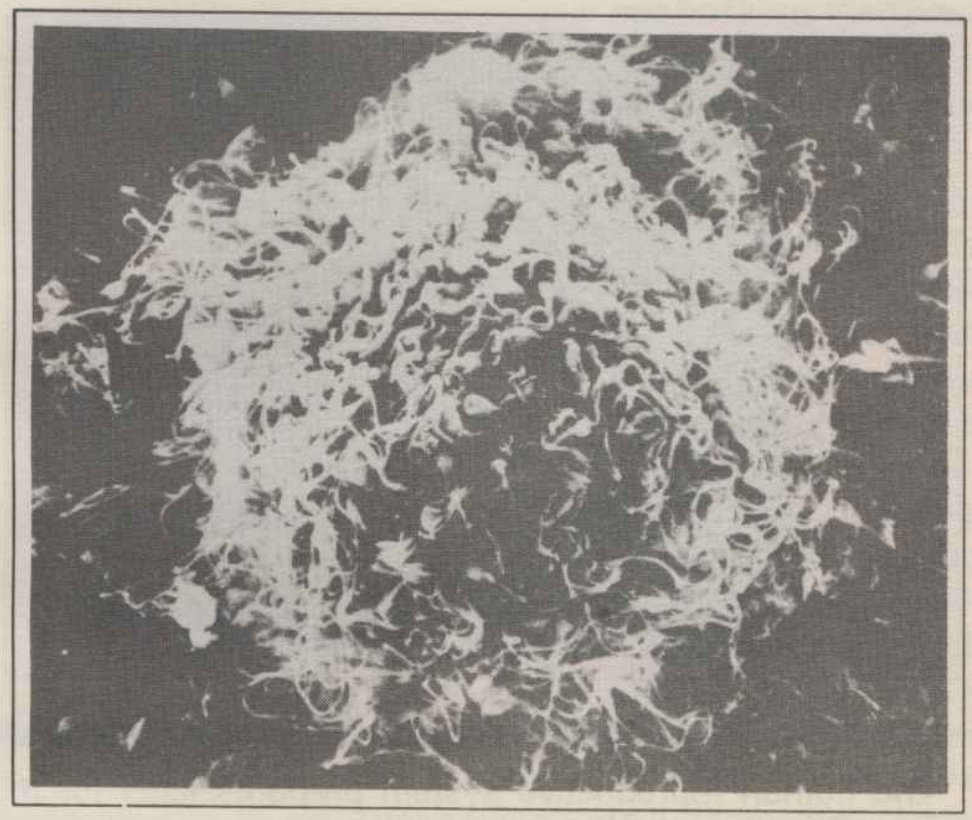
The important event is not division - that comes later; it is the sperm meeting the egg and fertilizing it. New light has been thrown on the process of this fusion with a rather startling finding being reported in a recent issue of the *Proceedings of the National Academy of Sciences, USA* by Dr. David Garbers of Dallas. Texas and Dr. Michael Eisenbach and associates of Rehovoth, Israel.

These scientists have discovered that the unfertilized human egg sends out a signal to communicate with the sperm. The signal, which is sensed by the sperm cells, triggers them to swim up the **fallopian tube** of the womb. One of them eventually meets the waiting egg, fertilizes it and this union ultimately leads to the creation of a unique human child. Quite a feat !

It has been known for a long time that the womb of the mother makes elaborate preparations for the grand event of conception to occur. This, of course, ends with the fertilization of the egg cell of the female by the sperm of the male. Beginning at the time of puberty and lasting until menopause, the female prepares for the event regularly. This cycle of reproductive readiness may occur over 400 times in the case of human females. New blood vessels, new tissues, and a tougher lining for the womb are produced and enlargement of glands occur, all in preparation for new life.

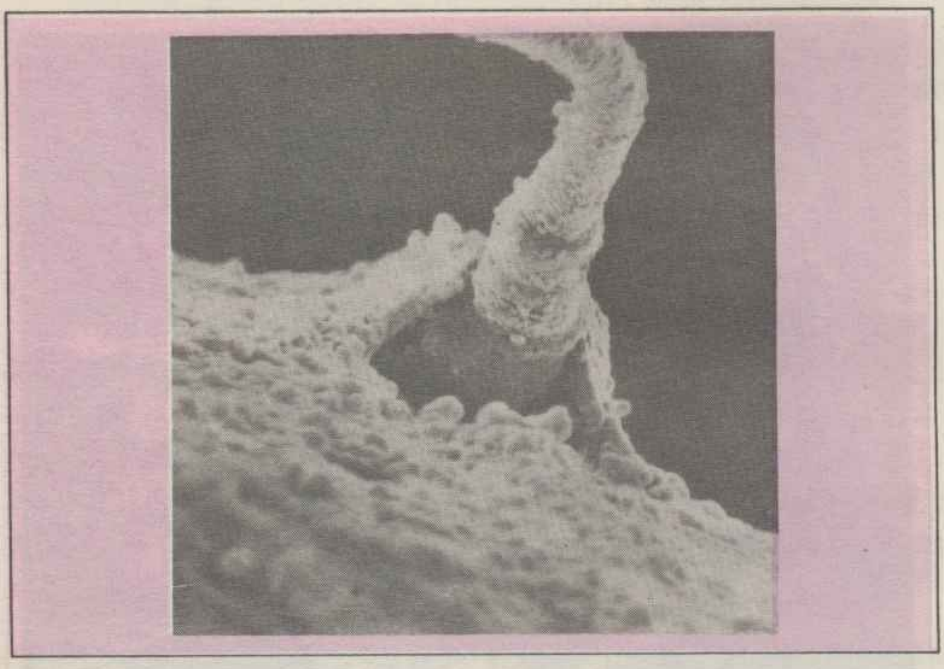
Around the middle of this preparatory cycle, one of the ovaries releases an egg—not a million as the male does with his sperms, not even a handful but a single individual one. It is this single egg that has the potential to grow into an offspring and thus to initiate a new generation.

But for that to happen, the sperm cell of the male should unite with this egg cell and deliver its share of chromosomes to those already present in equal number in the egg. The first cell that is formed by the union of the male and female cells is called the zygote. This fusion or fertilization leads to repeated cell division which results in the progressive growth



Millions of sperms flock to the egg

of the embryo. The term embryo, derived from the Latin word meaning to swell from within, is very appropriate. The sperm has to travel up the neck or cervix of the womb through the fallopian tubes to meet the lone egg and fertilize it. The route taken by the sperm often leads through hostile territory and the road winds uphill all the way. But this does not deter the sperms which blaze the trail rather overexcitedly, seemingly undaunted by the vaginal and uterine fluids flowing the opposite way. Like participants of the Boston Marathon, where thousands throng the starting line but few have the stamina to last till the finish, the sperms also jostle and wriggle their way to the egg. Of the about 280 million spermatozoa that start the trek only about a few hundred reach the upper tract of the fallopian tube. Of these hundreds, only one will fertilize the egg.

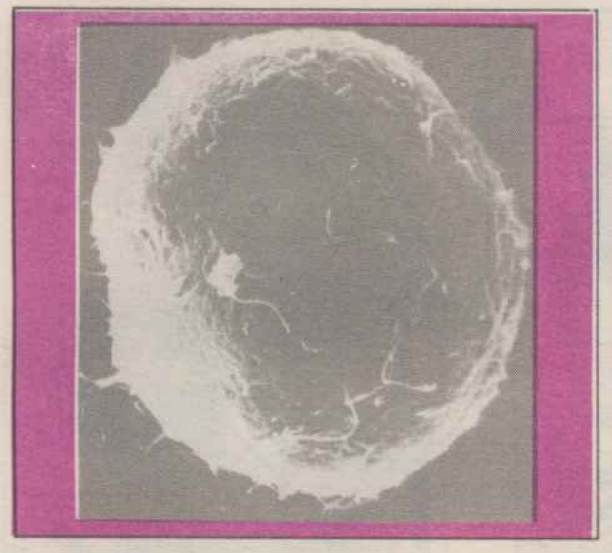


Winner takes all

The human reproductive system prepares itself for this great event in several ways. The male produces not just one sperm cell, but millions of them reinforcing the notion of strength in numbers. This enormous number increases the chances of one of them meeting the egg. The cervix aids their endeavour by coordinating the ovary. When the egg is released, the cervix lines itself with a mucous layer so as to smoothen the path of the sperm up the cervix and the fallopian tubes.

However, if fertilization does not occur and the ovum is not delivered to the womb, all the elaborate blood vessels, tissues and fluids prepared for the occasion are discarded in the form of the monthly period. Nature being patiently persevering, the process then starts all over again the following month.

The odds against the sperm finally meeting the egg are very high, and naturally every possible assistance, strategy and ploy that aids the fertilization of the egg is welcome. It is in this light that the Garbers-Eisenbach discovery, that the



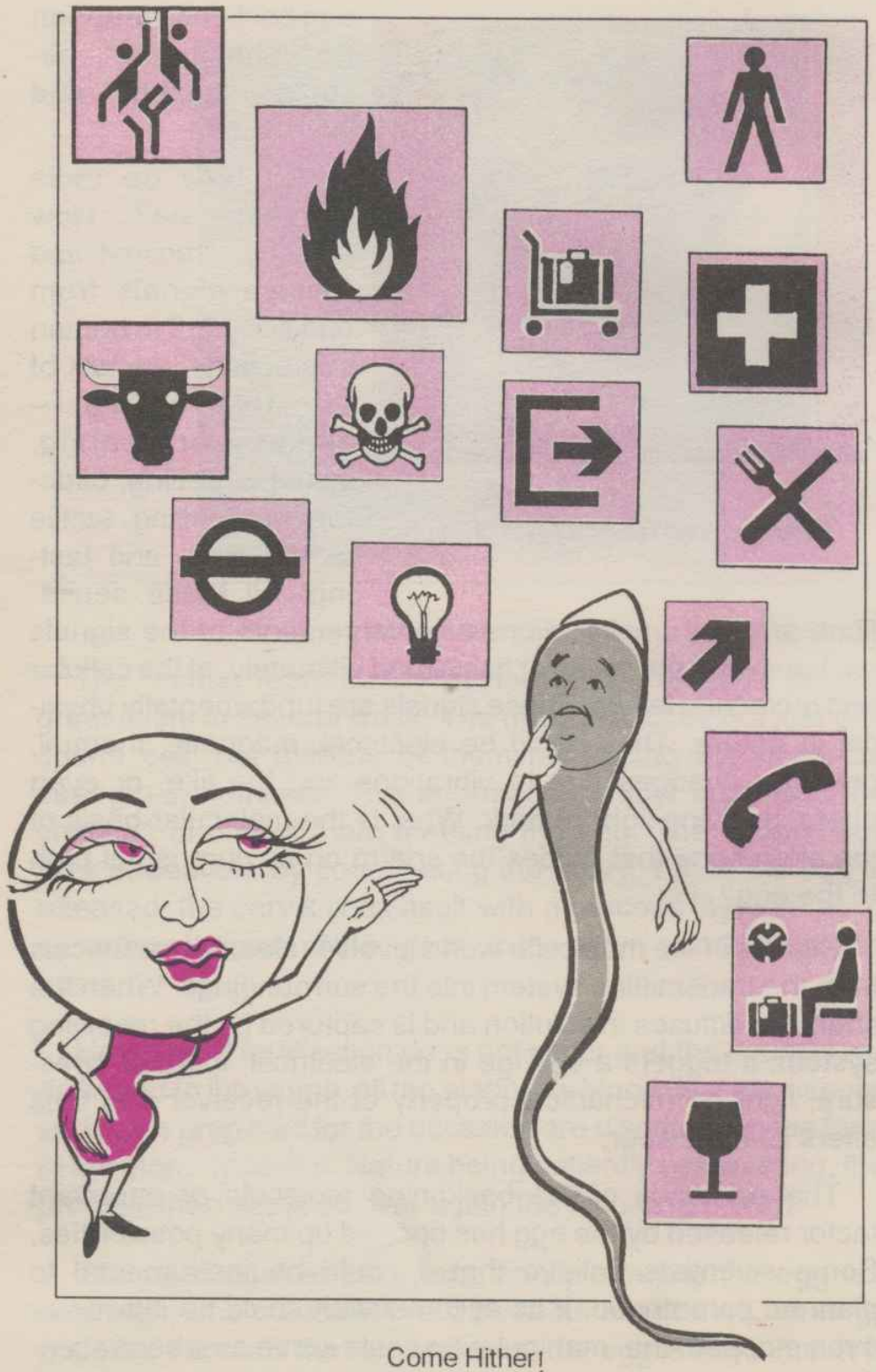
Fertilized egg ready for first division

egg beckons the sperm to "come hither", becomes insightful and interesting.

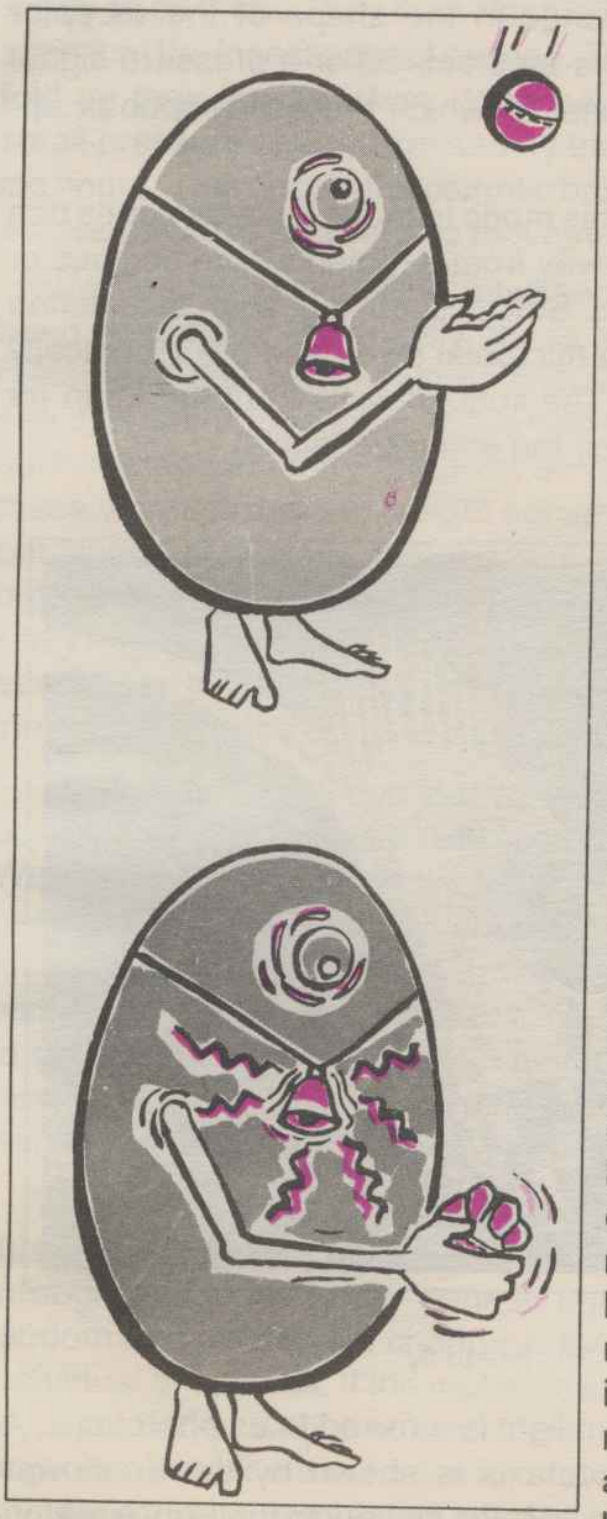
But how do cells communicate? How do they transmit and receive signals from one another? In human interactions, we talk of the five senses—auditory or hearing, visual or seeing, olfactory or smelling, tactile or touching, and tasting. All these sensations are just amplifications or enlargements of the signals exchanged at the organ or tissue and ultimately, at the cellular and molecular levels. These signals are fundamentally physical in nature. They could be electrical, magnetic, thermal, pressure changes due to vibrations and the like, or even photo involving light or dark. What is the molecular basis of the siren song that guides the sperm on its purposeful path to the egg?

Signals in the molecular world involve releasing chemicals from the transmitting system into the surroundings. When the chemical diffuses in solution and is captured by the receiving system, it triggers a change in the electrical, thermal, pressure, light or mechanical property of the receiver and thus alters its behaviour.

The existence of the beckoning molecule or attractant factor released by the egg has opened up many possibilities. Some scientists believe that it could be instrumental to planned parenthood. If its accumulation could be slowed or even stopped, the manipulation could serve as a contraceptive measure. Alternatively, the molecule on the sperm that



Come Hither!



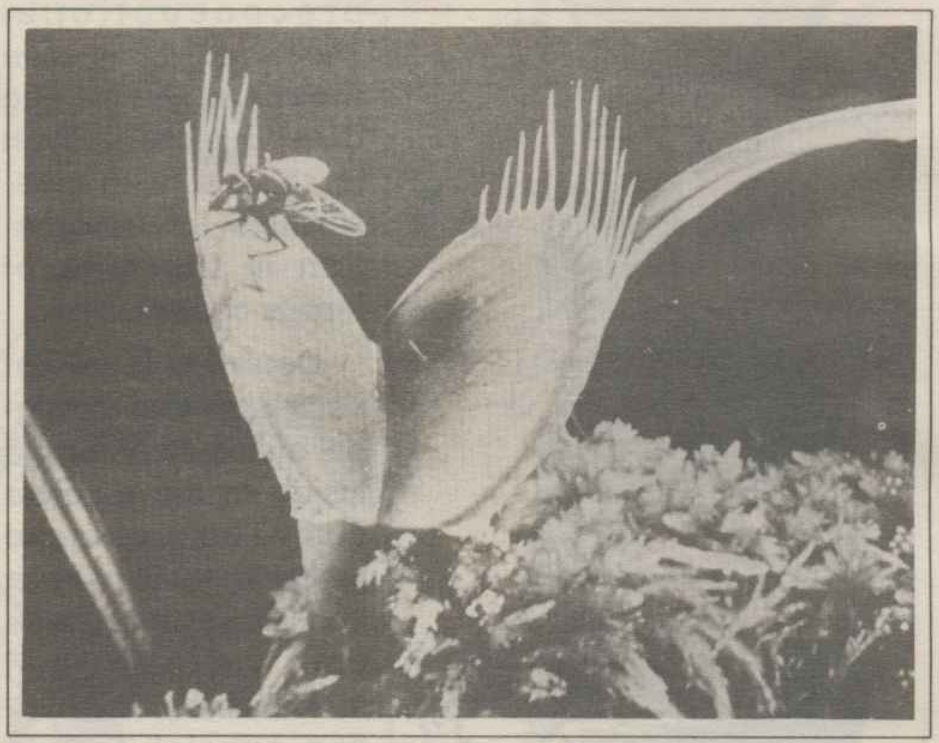
Receptor molecules alert the cell

specifically responds to this chemical from the egg can be blocked and the sperms dissuaded from moving towards the egg. Scientists are also wondering if infertile women have a low level of this factor. Clearly, the molecular manifestations of the preconceptional chemical talk between the sperm and the egg deserve more study. It is replete with possibilities for exploitation in the area of fertility control.

Usually, it is not necessary that the signal molecule enter the inside of the receiver cell. The preferred mode is to capture it knocking at the outside door of the cell membrane. The cell has special protein molecules embedded in its boundary membranes. These are called receptors because they grab the signal molecule and bind to it. The process

of binding causes a change in the shape of the receptor molecule and this triggers an electrical or a pressure signal across the cell membrane, to which the cell responds appropriately.

Many bacteria adopt this mode to move towards zones rich in nutrients or to move away from a region where noxious or toxic substances abound. Strictly speaking, such acts define "smart" behaviour at the microbial level, and are technically termed as chemotaxis (The suffix *taxis* is from the Latin for touching or feeling; hence the adjective tactile).



Too late to fly

Similarly responding to light is referred to as phototaxis. A striking example of phototaxis is shown by the sunflower plant, which literally turns its huge flowers to the Sun, tracking it in the sky, from east to west, as the day proceeds. Carnivorous plants such as the Venus fly trap feel the unsuspect-

ing fly sit on them. They then close in by moving the trapdoors such that the insect cannot escape. The "touch me not" plants fold up their leaves when touched. The signal here is the small pressure change caused by the sitting fly or the touching finger. This upsets the osmotic balance between the fluids across the cells and causes movement of the plant parts.

Words such as signalling, intelligence and attractants have been used here. Essentially, these are figures of speech or metaphors that betray human chauvinism. Life, as humans see it, is anthropocentric. It focuses only on humans. While such metaphors carry the point across, the danger in using these words is that they might convey a sense of purposeful behaviour or a goal orientedness in bacteria and plants, organisms that lack brains or even a central nervous system. They might also colour our judgement of the sperm which is essentially just a capsule of DNA and does not have any innate intelligence or motivation to behave as it does.

The theory of evolution repeatedly stresses that organisms do not evolve or "improve" with a set purpose towards a goal. We do not pass on what we learn during our lifetime to our generations through body chemistry. Many of the painful lessons to be learnt on the way to adulthood could have been eliminated had we been able to transmit lifetime experiences to our offsprings. These are at best passed through cultural means — books, folklore, legends, teachings, all "software," not wired into the genes that we pass on.

The only way a new trait or behaviour can be inherited is through the genes of the organism. That happens only through mutations in the genetic makeup. A mutation is a random and permanent change in the genetic makeup of an individual or species. If the mutation empowers the organism to cope better with the environment, then that particular organism has a better chance for survival and propagation as compared to its unmutated fellow members of the species. With time it is the lineage of the mutated individual that survives: the unmutated cousins who could not cope are

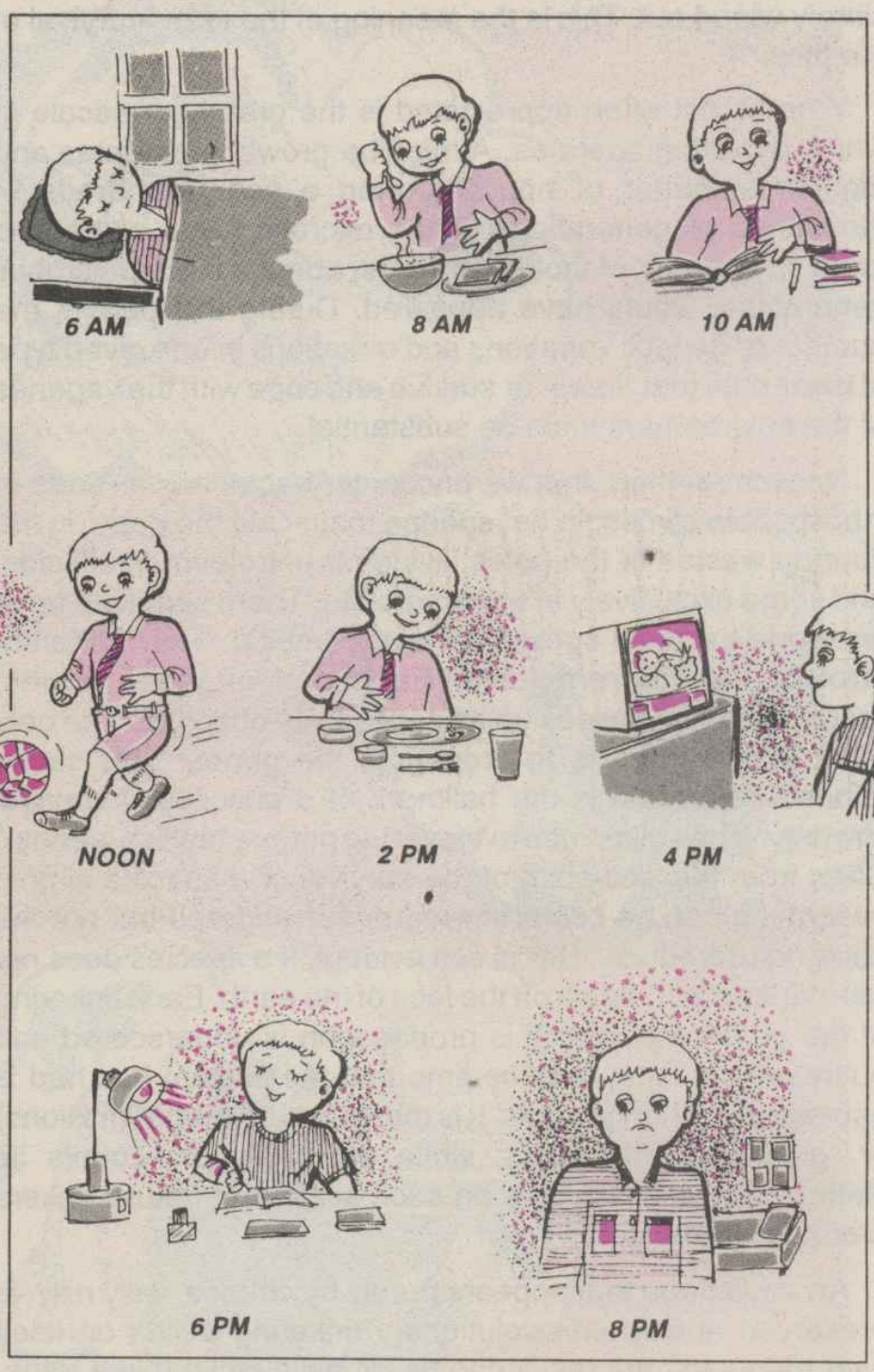
The software for transmitting knowledge has also evolved over the years

simply wiped out. This is the meaning of the term "survival of the fittest"!

What is not often appreciated is the grand time scale in which evolution operates. A microbe grows, procreates and dies in a matter of hours. During a year, hundreds of thousands of generations of the microbe have come and gone. In a matter of thousand years, about a billion microbial generations would have appeared. During this period, the number of genetic variations and mutations in one given type of bacterium that "keep" or survive and cope with the vagaries of the environment must be substantial.

No wonder then, that we encounter bacteria in all sorts of inhospitable climes, in hot springs that scald the body, in the spartan wastes of the poles, living off petroleum in oilfields, and some exclusively in salty marshes. There seems to be at least one bacterial species for every habitat. Truly, different strokes for different folks! Each one of these copes, propagates and passes on its genes to its offspring. The only thing that matters is to propagate the genes. This single minded obsession is the hallmark of a successful survival strategy. It has given rise to the telling phrase "selfish genes." Seen from the viewpoint of the survival of a species almost everything can be compromised or surrendered but not the ability to reproduce. This is self evident. If a species does not reproduce it will vanish off the face of the earth. Each tinkering of the genes that aids this propagation is underscored and buttressed in time, and the time that the system has had is geological and languorous. It is measured in "mega" (millions) or "giga" (billions) years, while the bacterium counts its lifetime in milli-years. It is on such scale that nature tinkers around with life forms.

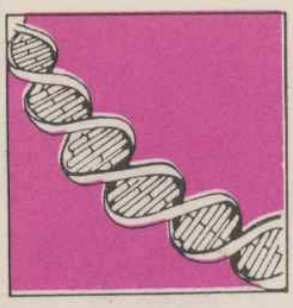
An advantage that appears purely by chance, willy nilly, is preserved and when evolutionary tinkering builds on itself over the years, the result can be unbelievable. It is a transformation from the original starting material that can be as exquisite as a piece of jewellery, or a wrist-watch fashioned




The number of bacteria double every half an hour

from a shapeless slab of gold. But the important difference is that the goldsmith planned and designed his artistic creation, while nature is the "blind watch maker". The gigantic scale of time, the colossal canvas of nature and the factor of sheer chance have created and shaped life. The same elements governed the conditions that made it possible for man to appear. The molecular "come hither" signal of the unfertilized egg to the sperm is just one such strategic step in this grand canvas of life.

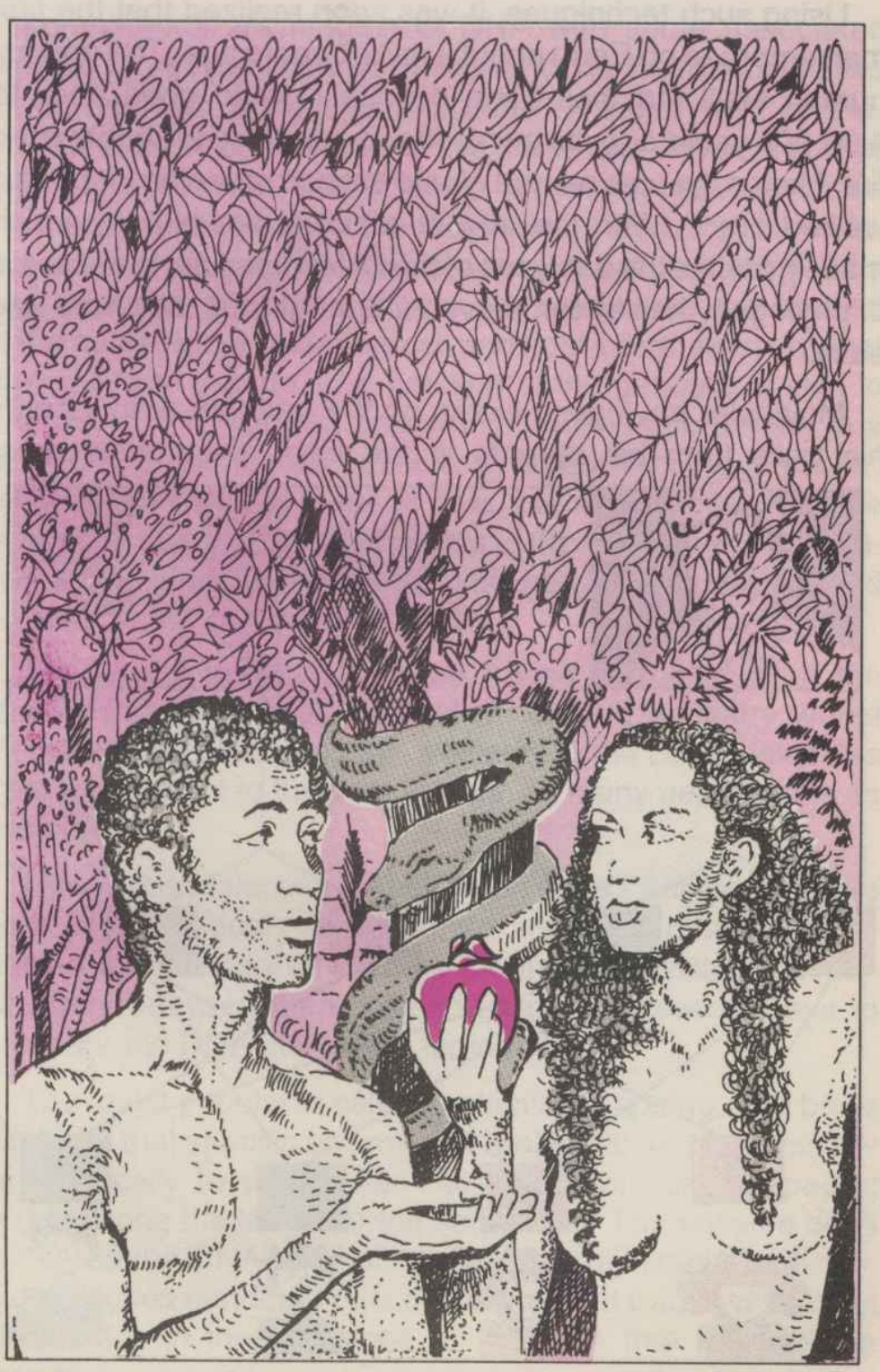
The long reign of man has been marked by curiosity or the desire to know. Man has always been intrigued by his ancestry. Each culture has myths and legends about its genetic heritage and lineage. The concept of *Gotra* in Hinduism traces families back in time to one person from whom the community descends. The *pandas* of Banaras and Puri have been smart enough to stoke the chauvinistic urge of many a pilgrim thus making a neat packet by claiming to read through records of visits by the ancestors of the present pilgrim.



## Adam and Eve

Christianity holds that the first man was Adam and that he was created in God's image. Likewise, Eve is held to be the first woman. All mankind is thus thought to have arisen from Adam and Eve. The Dogons of Mali in Africa believe that the Creator placed twin eggs in a plaited basket and let them fuse to produce the first couple.

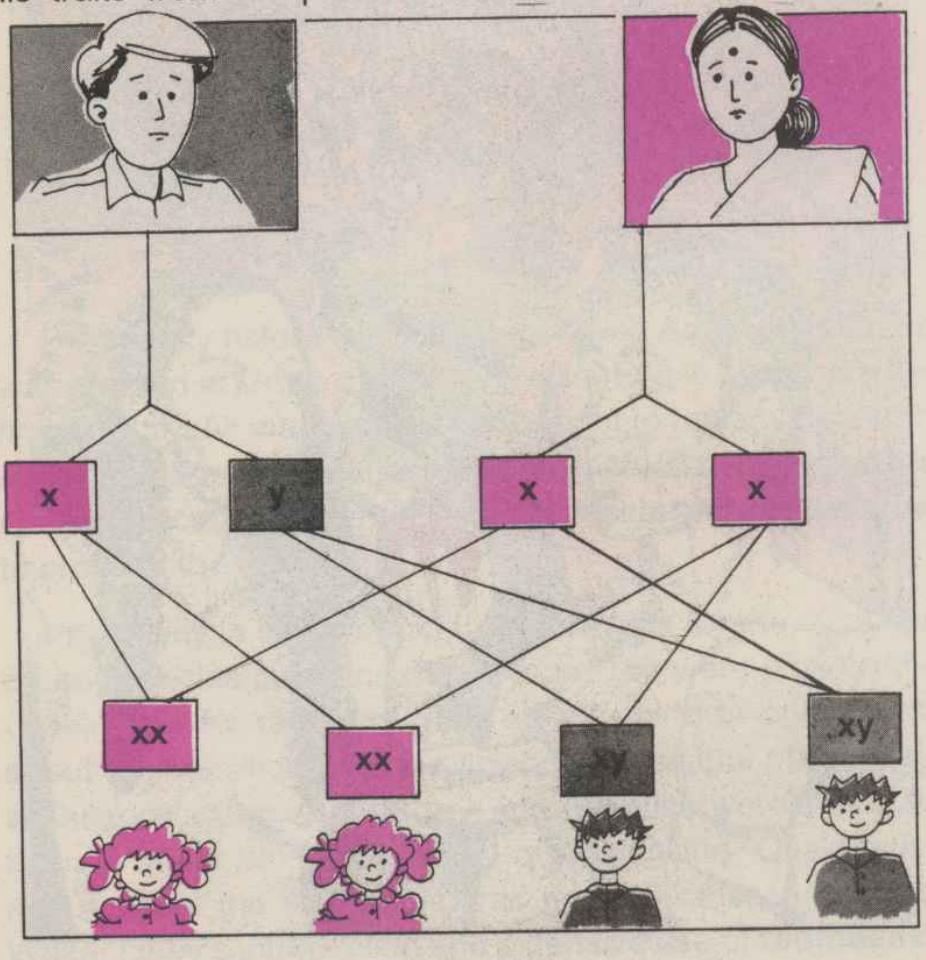
Phylogeny is the term used by scientists for the evolution of any species including that of man. The word *phylo* is the Greek term for race and *geny* means birth or origin. Until about a generation ago, the sciences of animal physiology, **anthropology**, **palaentology** and genetics were the main tools to study the racial history of humankind. Quantitative rigour about the time scale was added in the 1940s after Walter Libby's introduction and extensive use of **radioactive** decay of **isotopes** in materials. This has been used as an atomic clock to date specimens of historical interest.



The story of man's origins has many versions

Using such techniques, it was soon realized that the first man emerged on Earth about a million years ago. The earliest human skull that has been definitely dated goes back to 4,00,000 years ago. Its owner stood erect and probably used fire. Hence, he was named *Homo erectus* meaning 'man who walked erect'. With the advent of molecular biology which made possible the analyses of the DNA molecule, the science of phylogeny has become even more rigorous, accurate and easy.

Molecular phylogeny works on the fact that man inherits his traits from his parents in the form of chromosomes.



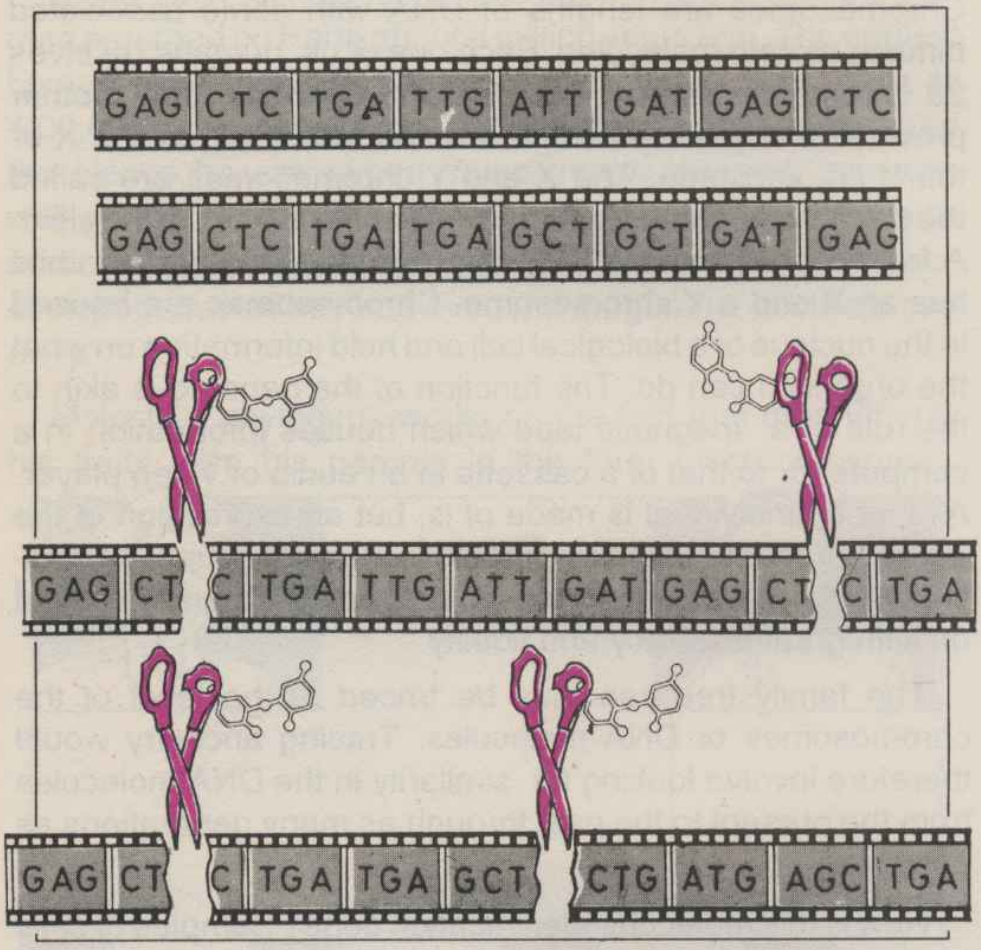

Boy or Girl?

Chromosomes are lengths of DNA with some associated binding protein molecules. Each one of us humans receives 23 chromosomes from each parent. Of these, the mother provides the X chromosome and the father either the X or the Y chromosome. The X and Y chromosomes are called the sex chromosomes for these determine the sex of the child. A female child has two X chromosomes while a male child has an X and a Y chromosome. Chromosomes are housed in the nucleus of a biological cell and hold information on what the organism can do. The function of the genome is akin to the role of a magnetic tape which houses information in a computer or to that of a cassette in an audio or video player. All that an individual is made of is, but an expression of the genetic matter donated by parents to the subsequent generation. DNA copies from parents to progeny are usually passed on with great accuracy and fidelity.

The family tree can thus be traced at the level of the chromosomes or DNA molecules. Tracing ancestry would therefore involve looking for similarity in the DNA molecules from the present to the past through as many generations as possible.

How is this molecular identification done? Samples of cells from two individuals are obtained, the DNA molecules extracted and compared and the degree of similarity or dissimilarity between them noted. There are several ways to compare the DNA molecules for identity.

The most popular is called restriction mapping. The basis for this is that specific enzymes or molecular scissors cut the long helically twisted thread-like DNA molecule at specific places along the length or the sequence. The enzyme does not shred the DNA to small bits of unrecognizable particles, but produces restricted cuts or well defined fragments at just those sites in the sequence of the DNA that the enzyme recognizes, binds to and hydrolyzes. The lengths and weights of these DNA fragments are estimated. This estimation is based on the distances to which they can move along a gel


GAG CTC TGA TTG ATT GAT GAG CTC
GAG CTC TGA TGA GCT GCT GAT GAG
GAG CT C TGA TTG ATT GAT GAG CT C TGA
GAG CT C TGA TGA GCT CTG ATG AGC TGA

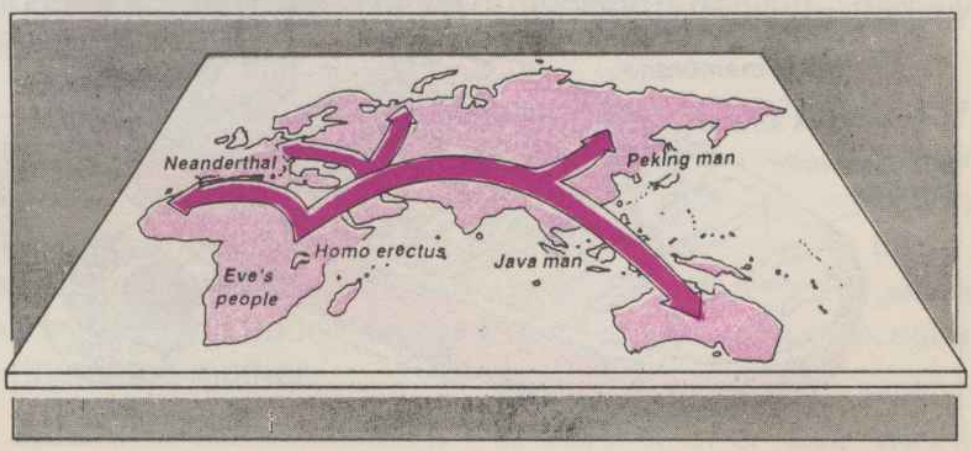
Molecular scissors cut DNA at specific sites

upon application of an electrical voltage. Each DNA fragment leaves behind its designer signature on the gel. This method is called gel electrophoresis.

The use of several such different restriction enzymes in the same DNA produces a restriction map on the electrophoresis gel. These look like the bar code lines that one sees in the back of paperback books, which are recognized by a laser reader in the cash register. Two DNA molecules that are identical or nearly so will produce the same or very similar bar code diagrams or restriction maps. The closer the DNA match, the more similar is the map. Thus, there is a method

to compare the DNA heritage of individuals and to calculate how they are related through generations. The restriction map of the DNA of a child will match the mother's and father's, since the child has inherited its DNA exclusively from these two. It will also show some similarity with the DNA maps of cousins, grandparents and other relatives but will be distinctly different from that of an unrelated stranger. Disputed paternity cases can thus be resolved using such DNA-matches. And now even race horses are to be routinely checked for DNA matches with their supposed dames and sires. Racing enthusiasts hope that this will establish the lineage of the thoroughbreds beyond doubt.

Can we then trace the genealogical tree back to the first ancestral pairs? If we identify the DNA inherited exclusively from the mother, that would give us matrilineal genealogy. Likewise, if a male-specific DNA can be identified and its restriction map analyzed over generations, we can identify the patrimonial line. The current excitement in molecular genetics concerns this aspect of DNA matching. Since there is DNA that can be identified as being specifically inherited from the mother or the father over not just a few generations, but millions of years, it is little wonder that molecular biologists are ecstatic about their chances of going back to the dawn of

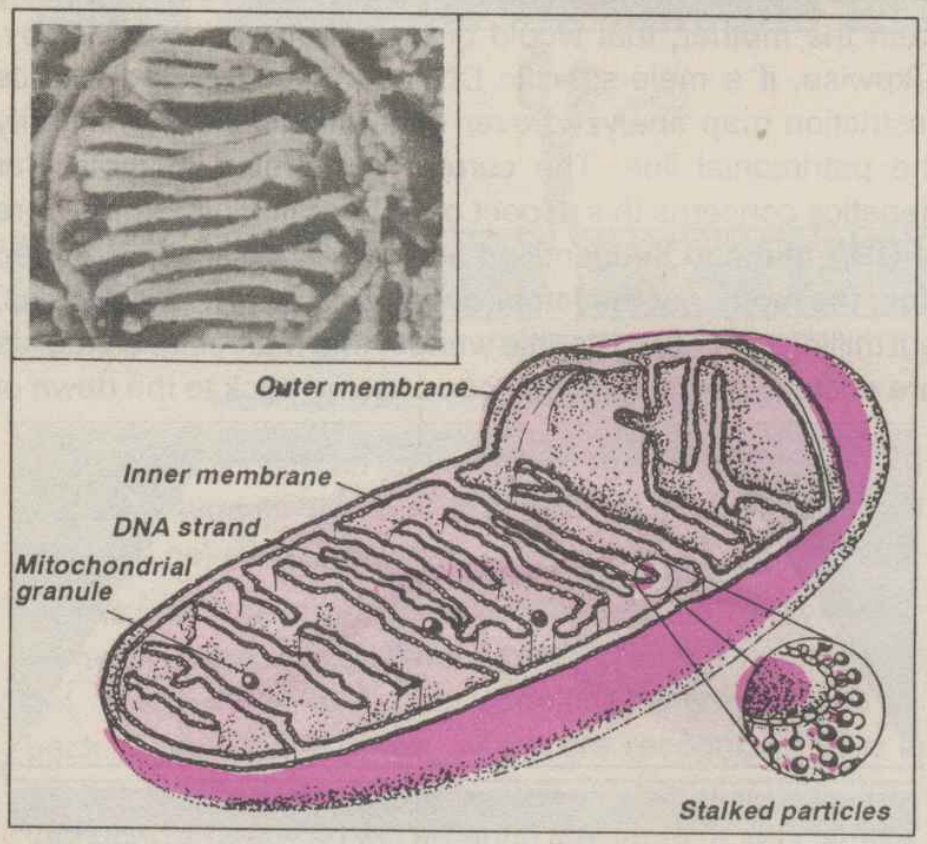



Following Eve's footprints

the human species and following the species as it colonized the world.

Thirty years ago, Linus Pauling and Emile Zuckerkandl of the California Institute of Technology (USA) showed that biological molecules such as proteins and DNA could be used as evolutionary clocks. The more mutations that accumulate in a specific protein or DNA sequence, the older is its lineage. The line branches at every point of divergence from the parent sequence. Thus, it should be possible through analyses of contemporary human DNA to construct an evolutionary tree. This would reach right back into history to the first man and first woman— call them Adam and Eve if you will.

Think for a moment what the father gives to his progeny and what the mother gives. The father gives his child only his



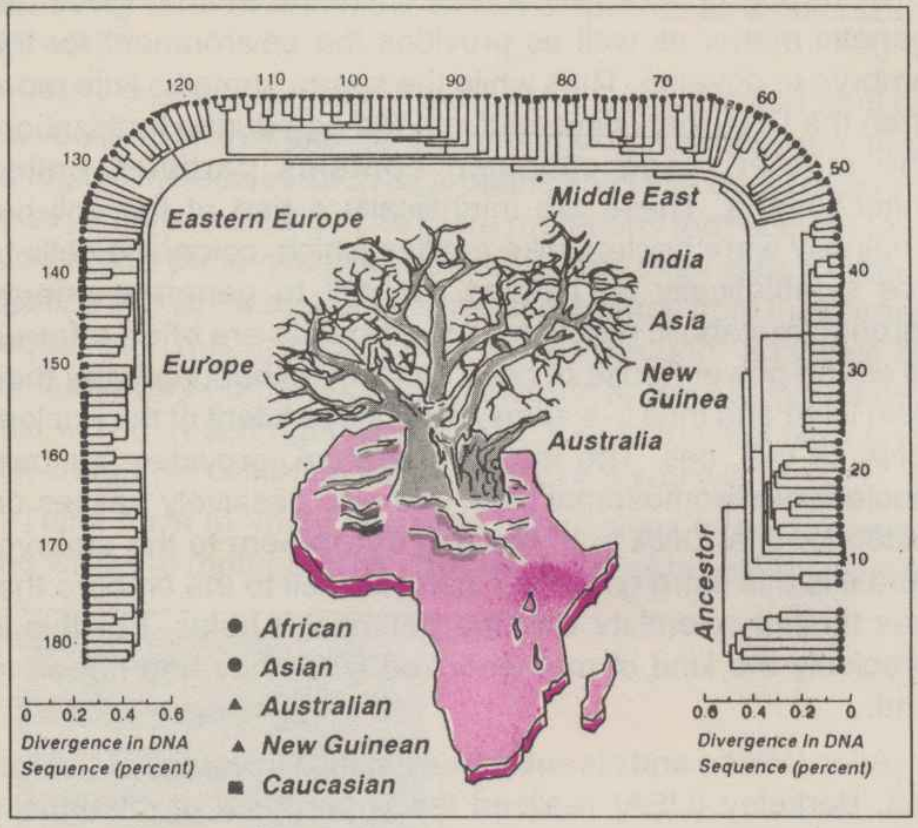

Mitochondria; cell organelle and clue to the past

DNA through his chromosomes while the mother gives her genetic matter as well as provides the environment for the embryo to develop. Thus while the sperm contains little more than the DNA that is injected into the egg during fertilization, the mother's egg cell also contains particles called mitochondria. These are intrinsically a part of the cell but originally were bacteria-like entities which colonized cells to live symbiotically by helping the cell to generate energy through metabolic reactions. Mitochondria are often referred to as the power house of cells. Now, mitochondria carry their own DNA and thus live somewhat independent of the nuclear DNA of the cell. The mother therefore, provides her own nuclear or chromosomal DNA and also passively passes on mitochondrial DNA from her egg **cytoplasm** to the embryo. And it is this extra gift of the maternal cell to the embryo that has thrilled scientists with the potential it holds. For this is precisely the kind of mother-linked DNA they had hoped to find.

Allan Wilson and his colleagues at the University of California, Berkeley (USA) realized the importance of this strictly maternal inheritance of mitochondrial DNA or mDNA. They argued that if one were to generate restriction maps of the mitochondrial DNA from individuals all over the world and build a genealogical tree it would finally lead to the original mother from whom all or much of mankind is derived—namely "Eve"!

The researchers collected mitochondrial DNA from 147 people, drawn from Africa, Asia, Australia, Europe and New Guinea. They reacted the DNA with 12 different restriction enzymes and compared the restriction maps. Using these, they constructed the evolutionary tree from every divergence in the map, and traced it back in time to the original ancestor or the prime mother.

The answer was startling. The first mother or "Eve" was a lady from Africa who lived around 2,00,000 years ago. This figure is based on the estimated rate of divergence in




A phylogenic "tree" based on mitochondrial DNA

mitochondrial DNA sequence over the years. But how did the scientists pinpoint Africa? This was because Africans seem to have the highest mitochondrial DNA diversity indicating a long evolutionary history.

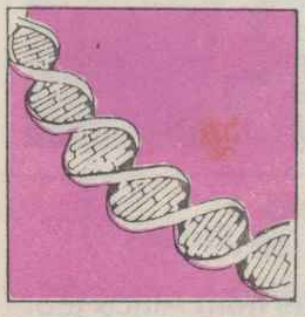
Who then was the First Father ? Who was "Adam"? Dr. Gerard Lucotte of the College de France in Paris reckons that the answer to this lies in the Y chromosome which, is a chromosome strictly associated with the male in normal humans. In fact, the American geneticist David Page has argued that, theoretically, the Y should serve as a mirror image of the genetic variations in mitochondria that led to "Eve". This is so because there is one part of the Y choromosome that has remained relatively unmutated over the ages. Dr. Lucotte collected blood samples from people of

many races and isolated the Y chromosomes from them. He used special diagnostic devices that detected restriction fragment variations in DNA of the chromosome. From the data obtained, he generated an ancestral profile which resulted in a remarkable finding. The most likely ancestral father figure might well be an Aka tribe pygmy from the Central African Republic. So, "Adam" was from Africa too!

'Father', dear Father

It is estimated that Adam too lived around 2,00,000 years ago. However, this dating is not based on any rigorous information on the divergence rate of the Y chromosome but is an educated guess, attempting to make him contemporaneous to "Eve". Classical anthropologists however feel that the "Garden of Eden" was not where Lucotte proposes but that it had a far more easterly location along the Rift Valley of East Africa.

From the idyllic Garden of Eden to the Vale of Tears, mankind has trekked a long route. He has battled pests, pestilence and disease on his way and triumphed, although at times at a heavy cost. In the early days of civilization life could be 'nasty, brutish ... short' but with time the quality of life has improved. Many diseases have been banished while others are on the verge of being conquered. The first step towards vanquishing them is a complete understanding of what causes them and how the etiology unfolds.

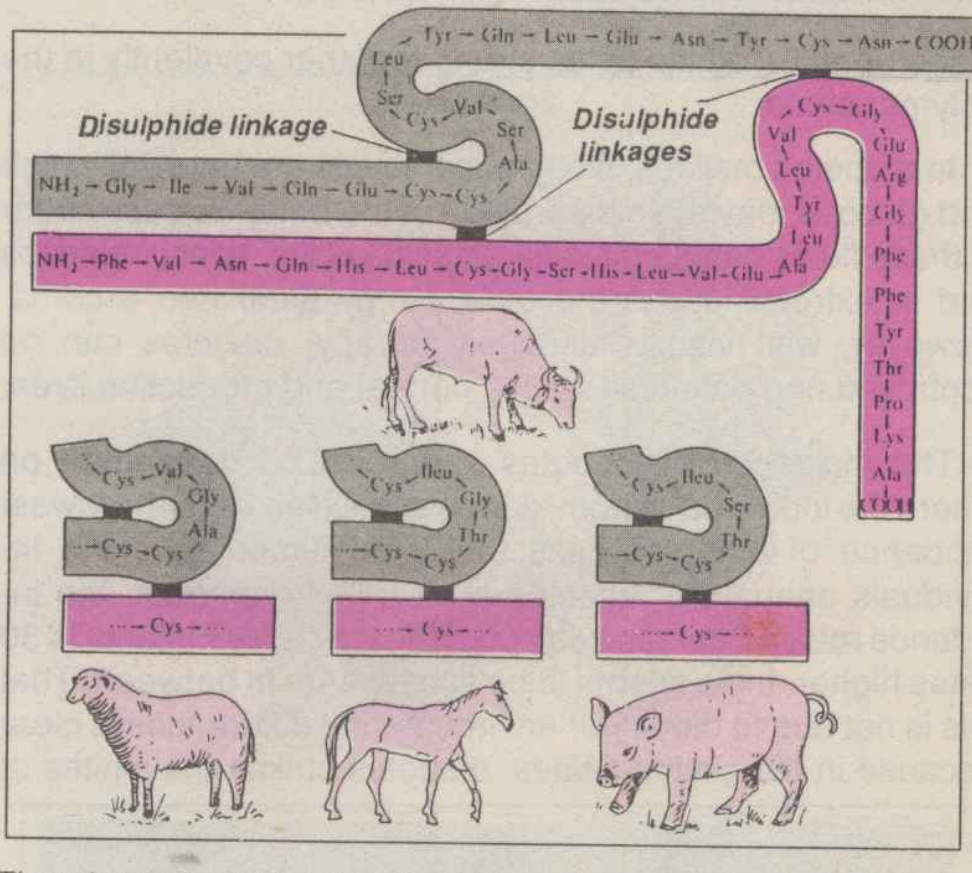


# New genes for old — and new genes for ever

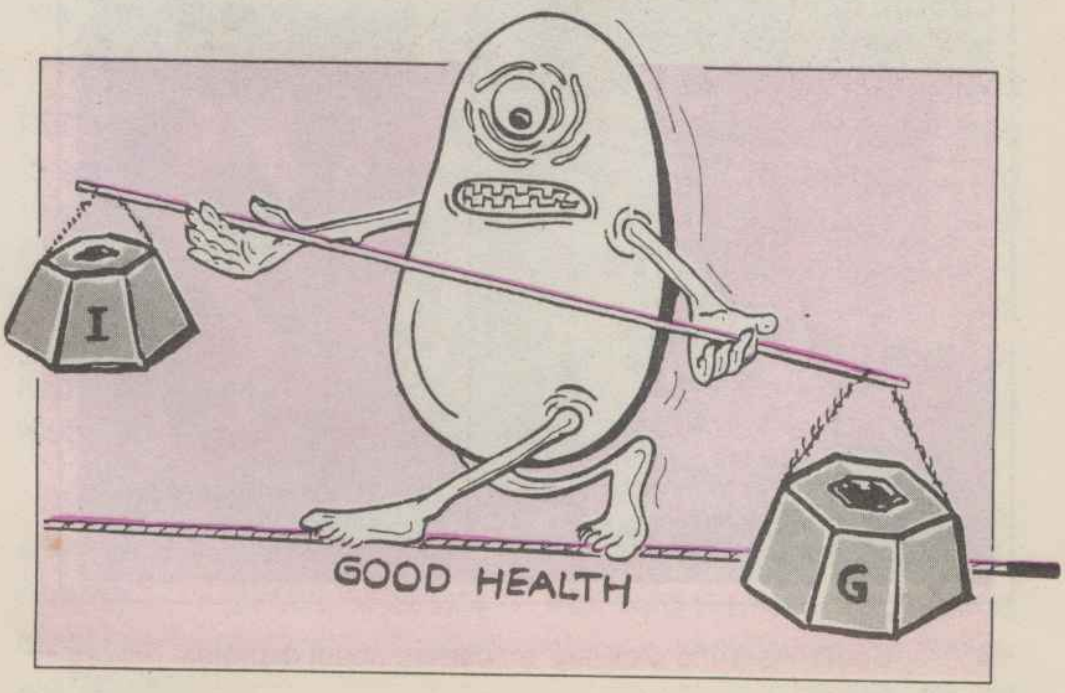
Many chronic diseases show marked geographic variations in their incidence. This is referred to as epidemiology, which refers to their dependence on hygienic factors, climate and other environmental features. Malaria and dysentery are two typical examples of such diseases which occur where poor sanitary conditions prevail.

It is easy to identify the factors that cause infectious diseases to spread rapidly. However, the geographic variation seen in noninfective diseases poses a challenge. Diabetes, or more properly insulin dependent diabetes mellitus (type 1 or IDDM for short), is one such chronic and systemic disease.

This type of diabetes is invariably associated with faulty carbohydrate metabolism. This is mainly due to a lack of insulin, a hormone secreted by certain cells of the pancreas, which regulates the metabolism and clearance of glucose from cells. The insulin molecule is a relatively small protein,



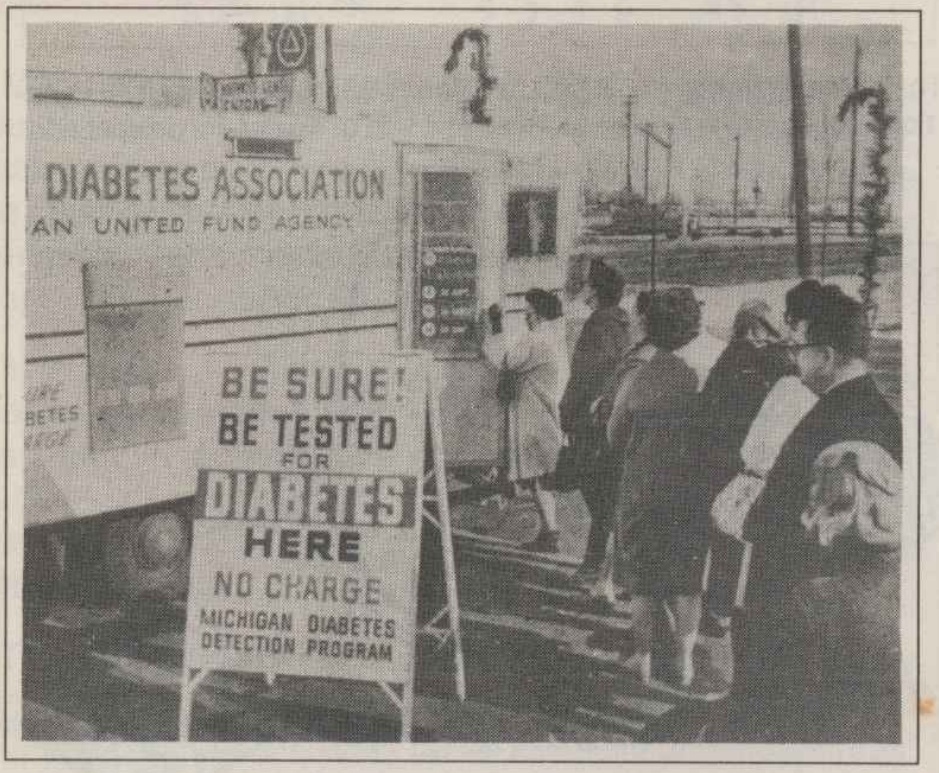

The primary structure of insulin of different species shows slight variations in the amino acid sequences at a fold of one of the polypeptide chains



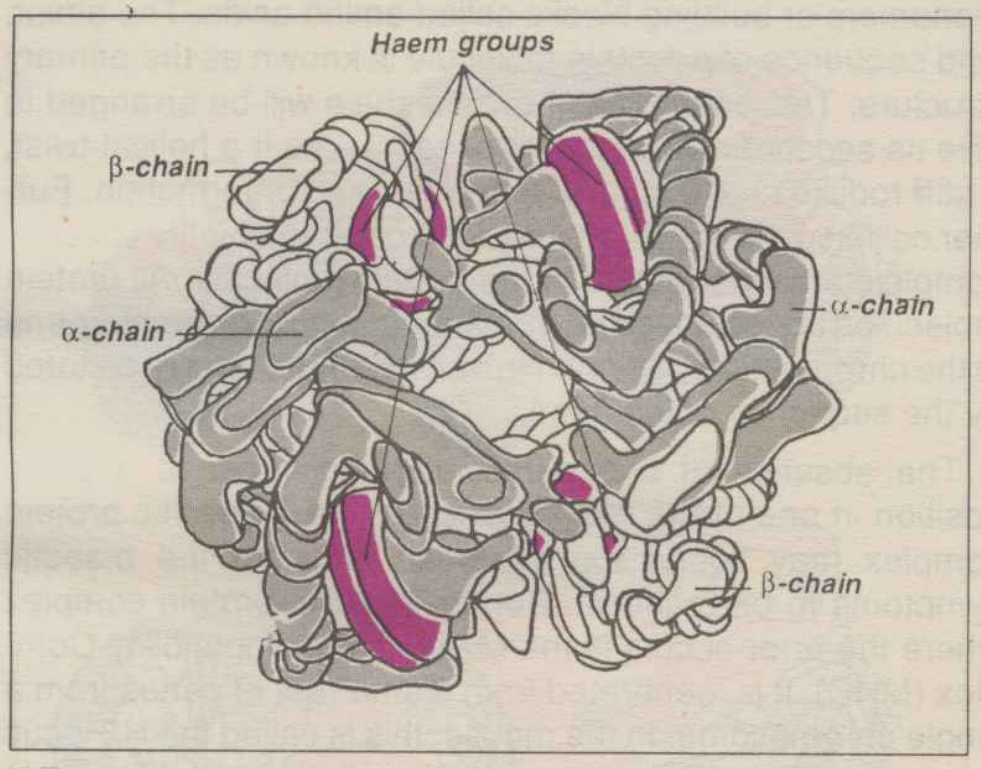
made up of 51 amino acids strung together covalently in the polymer chain.

In diabetes mellitus, blood sugar levels are unusually high and glucose may even be present in the urine. Excess sugar in these fluids can result in giddiness, loss of consciousness and in extreme cases the patient may enter into a coma. However, with insulin taken as therapy, diabetes can be controlled and patients can live normal and productive lives.

The incidence of diabetes seems to be dependent on where the individual is from. Japan and China have the lowest incidence of diabetes: less that one person per lakh individuals annually is affected by IDDM. In contrast, the incidence rate in Scandinavian countries, notably Finland, is 30 times higher. India seems to be somewhere in between. That this is not due to dietary or environmental differences is clear because in the United States diabetes strikes the whites at




Countries world wide are concerned about diabetes




Haemoglobin model

bout twice the rate among the blacks (20 as against 10 per lakh each year). Genetic differences within the populations may be the reason for this variation.

This does not raise eyebrows in medical or scientific circles because genetic predisposition or vulnerability to a disease is well-known. In this case, the excitement is about the finding that the incidence of IDDM is associated with the absence of one particular amino acid at a given position in one of the molecular chains of a specific protein complex. The gene that makes this protein is mutated leading to the error in metabolism. Truly reminiscent of "for want of a nail a kingdom was lost."

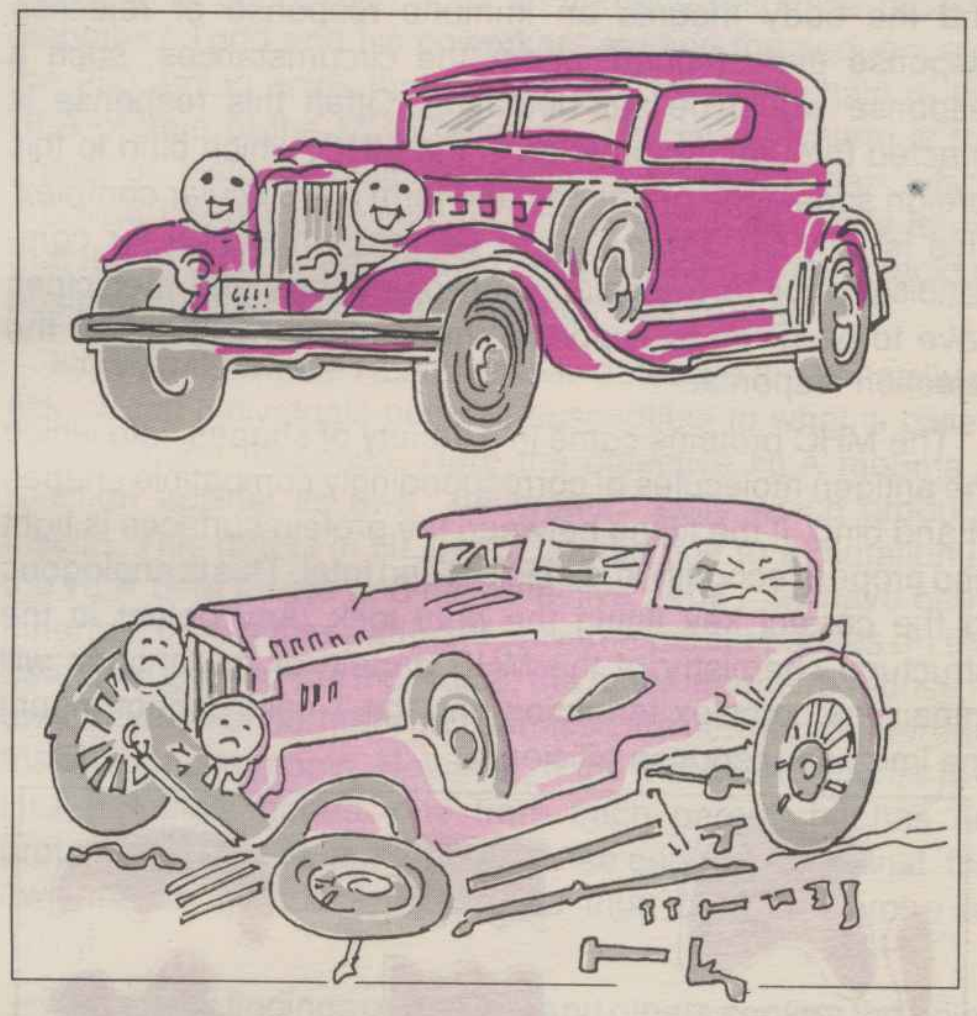
Proteins are very widely distributed biological molecules, which have many specialized functions. Enzymes, hormones, the fibrous structure of hair and the haemoglobin in blood are all proteins. They derive their name from the Greek word meaning 'first place'. Proteins are made up of

monomers or building blocks called amino acids. The amino acid sequence of a protein molecule is known as the primary structure. This determines how the chain will be arranged to give its secondary structure, or chain — be it a helical twist, a stiff rodlike shape or a wet, noodle-like conformation. Further coiling of the chain and packing gives the highly specific, complex tertiary structure of the protein molecule. All protein molecules are synthesized by instruction from a specific gene in the chromosome. Their amino acid sequences are dictated by the sequence of the gene.

The absence of one particular amino acid at a given position in one of the molecular chains of a specific protein complex may sound too minor an error for the diabetic symptoms to be manifest. But the specific protein complex where the error occurs is the Major Histocompatibility Complex (MHC). It is generated from a small set of genes from a single chromosome. In the mouse, this is called the H2 locus on chromosome 17, and in man, it is called the HLA locus on chromosome 6. Within this segment lie many genes that govern the immune response. Besides, even if a single amino acid is but a letter in the protein language alphabet, its importance cannot be overemphasized, for even a minor error can have major consequences.

Dr Janice Dorman and associates of the Departments of Epidemiology, Preventive Medicine and Pediatrics of the University of Pittsburgh in the USA have reported that a single, solitary error in the gene responsible for making the HLA-DQ beta chain of the 'MHC' makes the individual susceptible to type I diabetes. The situation is akin to one missing gasket or a minor nut in the engine causing the breakdown of a motor car.

Dorman and coworkers had collected blood samples of Chinese, American blacks and Caucasians, Norwegians and Sardinians, isolated DNA and analyzed these gene molecules for specific changes. In particular they looked for those changes that might have occurred in the HLA-DQ chain

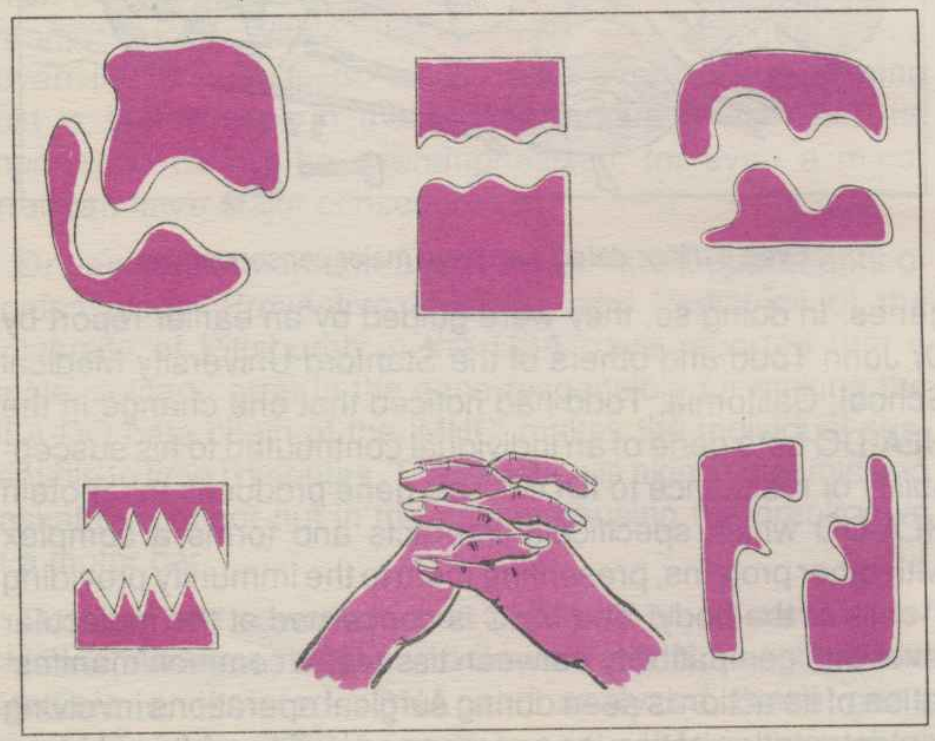


Even a minor defect can have major consequences

genes. In doing so, they were guided by an earlier report by Dr John Todd and others of the Stanford University Medical School, California. Todd had noticed that one change in the HLA-DQ beta gene of an individual contributed to his susceptibility or resistance to IDDM. This gene produces the protein HLA-DQ which specifically interacts and forms a complex with other proteins, presenting them to the immunity providing T-cells of the body. The MHC is concerned at the molecular level with compatibility between tissues. A common manifestation of its action is seen during surgical operations involving transplantation of tissues or organs as in skin grafts or kidney transplants. The donor tissue is seen as foreign or non-self

and the body mounts an immune response or rejection response even though, under the circumstances, such a response would be self-defeating. Often this response is enacted through the proteins of the MHC, which bind to this foreign substance or antigen and form a molecular complex. This is why an organ has to be tissue-matched for compatibility prior to transplantation operations and medicines have to be given to suppress the immune response or the rejection response.

The MHC proteins come in a variety of shapes, into which the antigen molecules of correspondingly compatible shapes fit and bind. If the fitting between the protein surfaces is tight and proper, recognition is efficient and total. This is analogous to the correct key fitting the right lock. Any defect in the structural chemistry of the MHC protein or the antigen will impair the complex formation and the T-cells cannot mount the immune response efficiently.



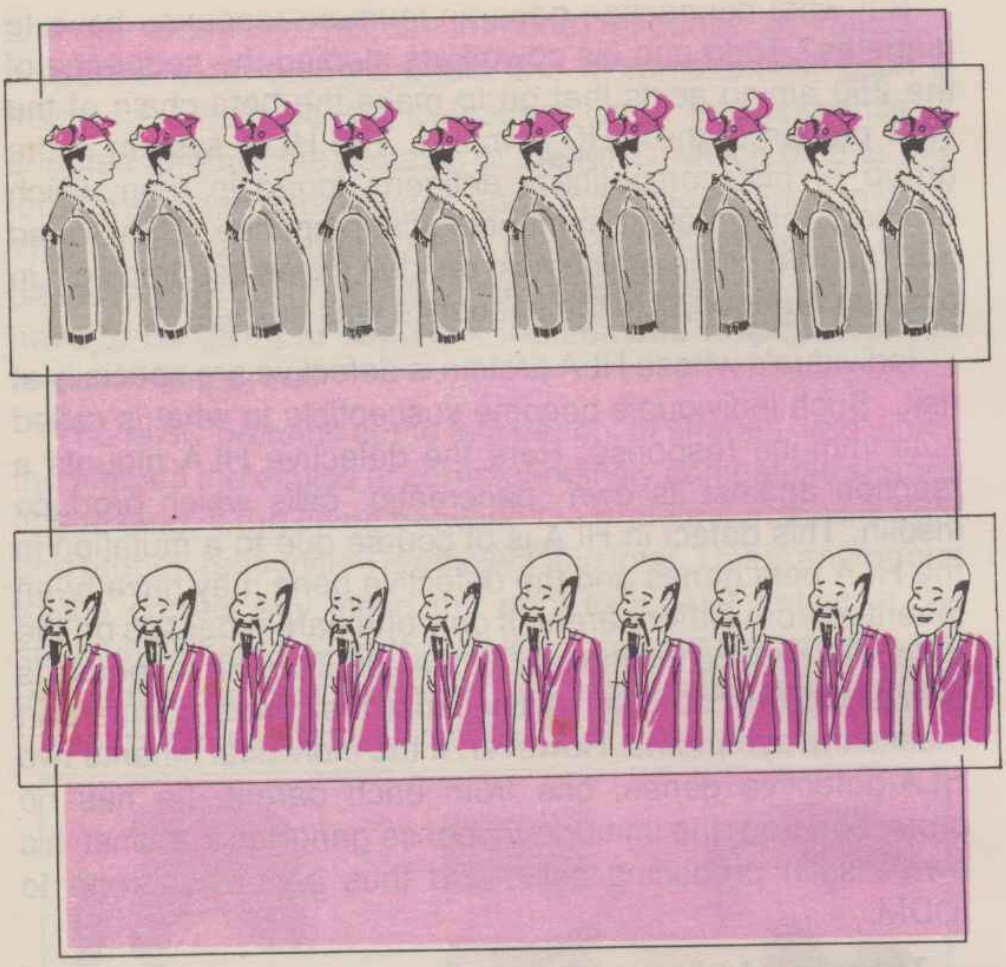
Compatible shapes fit and bind

But what connection does an immune response have to diabetes? Todd and his coworkers studied the sequence of the 250 amino acids that go to make the beta chain of the HLA protein of the MHC complex. The HL-A system is the group of histocompatibility antigens found in man which promote the greatest immunological response. They found that the role of the amino acid residue called aspartic acid in position number 57 in the sequence was crucial.

Individuals whose HLA protein is defective are specially at risk. Such individuals become susceptible to what is called auto-immune response. Here the defective HLA mounts a reaction against its own pancreatic cells which produce insulin. This defect in HLA is of course due to a mutation in the HLA beta genes and the defective gene may have been inherited from either parent. If only one parent passes on the defective gene to the individual, the autoimmune response is only a hidden or dormant trait because the normal counterpart masks the symptoms. However, if the individual inherits two HLA-defective genes, one from each parent, he has no protection from the immune response generated against his own insulin producing cells, and thus becomes prone to IDDM.

The recent findings of Dorman and others confirm the point made by Todd. The frequency of occurrence of the non-D57 HLA genes correlates well with the incidence of IDDM worldwide. The frequency of occurrence of non-D57 genes among Norwegians is six times higher than among the Chinese. This is in keeping with the relative rates of incidence of IDDM among them. This finding also holds out the possibility of testing for non-D57 genes in a new population and to predict the incidence of IDDM there.

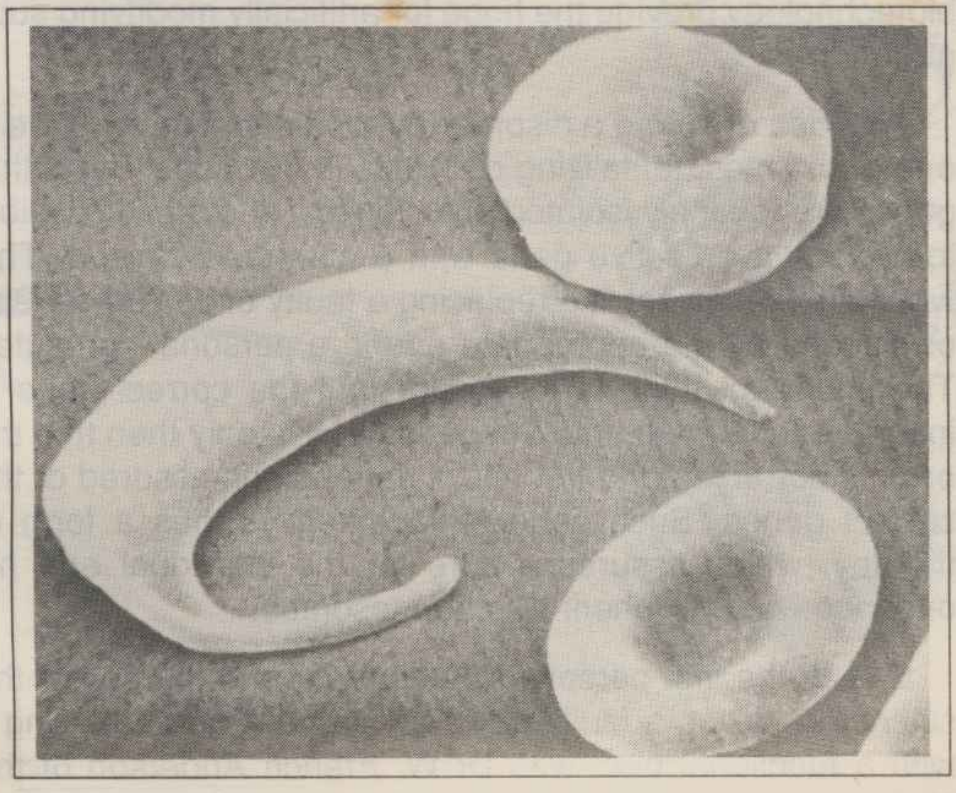
But why is aspartic acid so crucial to position 57 of the HLA beta protein? The detailed shape or the internal architecture of the molecule has been determined from analyzing the **X-ray diffraction** pattern of its crystals. There is a particular cleft in a specific region of the molecule where aspartic acid



The frequency of non-D57 gene is six times higher among Norwegians than in the Chinese

with its negative electric charge is located. The shape of the cleft and the positioning of the charge of the D57 there, seem to be the vital features. When D57 is replaced, the charge is lost and the shape altered. The precise fit between HLA and the peptide is compromised. This leads to a reaction cascading from the molecular to the organism level as an autoimmune response which leads to diabetes. It is ironical that the loss of aspartic acid should cause diabetes! Aspartic acid is abundant in young sugarcane and in sugar beets, while in diabetes the individual cannot absorb and metabolize sugar efficiently.

Another disease caused by the replacement of a single charged amino acid in the protein chain occurs with sickle cell anaemia. In sickle-cell anaemia, the red blood cells are distorted into crescent like shapes instead of the normal flat, disc like appearance. Scientists have found that the aminoacid, glutamic acid at position 6 in the beta chain of normal haemoglobin is replaced by the electrically neutral valine in sickle-cell haemoglobin. This change leads to reduced levels of solubility of the altered haemoglobin. As a result, the molecules stick to one another and show reduced affinity to bind to oxygen. This sticking together also distorts the shape of the cell which houses them. Cells containing normal haemoglobin are of the conventional doughnut shape. Those containing the mutant haemoglobin adopt curved sickle-like shapes, which hinders their ease of flow in the blood plasma. Sickle-cell anaemia is thus, another molecular



Sickle shaped erythrocyte (centre) compared to normal

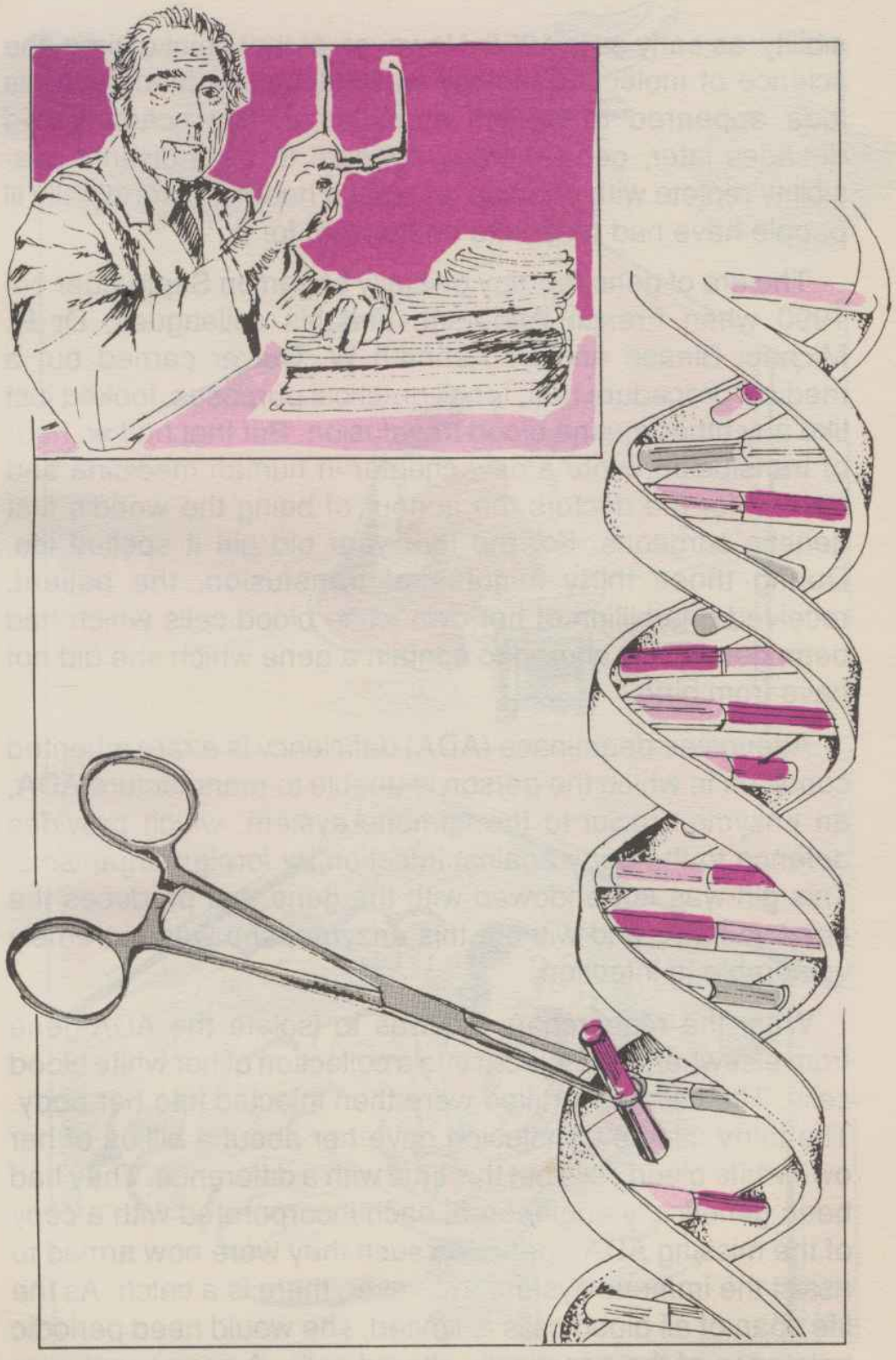
disease of genetic origin. The aminoacid replacement is the result of a mutation in the DNA molecule that codes for the synthesis of one of the haemoglobin beta chains.

About 150 different kinds of mutant haemoglobins have been found in humans. An abnormal haemoglobin is found in 1 out of every 10,000 individuals. Some of these can be lethal while others restrict some of the physiological functions of the affected individual. Mutations such as these are not limited to haemoglobin; but scientists think that all types of proteins in a given species are susceptible to mutation. Verily, we are, what our genes are.

What can be done about such susceptibility to mutations and its consequences? First, the chances of suddenly acquiring one is remote because DNA is a stable molecule. Mutations are normally detected in the cell and repaired by special enzymes present there. Secondly, current advances in biotechnology provide the hope for artificially modifying such gene errors.

The idea of curing a disorder by repairing a faulty gene is one of the most tantalizing concepts in medicine. Now that genes are routinely isolated and cloned it should be possible to replace a defective gene with a corrected version. This would be somewhat like replacing a faulty or corrupted tape or diskette by the appropriate one in a personal computer. The important thing is to ensure that the correct gene is incorporated forever into the genome. It is only then that the error is corrected and the future generations assured of the correct genes. Such gene replacement is thus a form of therapy, which assures a cure for the individual and his offspring who will inherit his chromosomes.

One of the first persons to dream of gene therapy or the science of taking a faulty gene out of a cell and replacing it with a functional one, was Dr W. French Anderson of the National Institute of Health in Bethesda, Maryland, USA. French Anderson's imaginative articles hinted at this pos-



Dr W. French Anderson; pioneer extraordinary

sibility as early as in 1968. However, at that point of time, the science of molecular biology was still in its infancy and his idea appeared to be too speculative. But scarcely two decades later, gene therapy appears to be a distinct possibility replete with promise; at least a handful of terminally ill people have had reason to be thankful for it.

The era of gene therapy officially began on September 14, 1990 when French Anderson and his colleagues, Dr R. Michael Blease and Dr Kenneth W. Culver carried out a medical procedure that, for all practical purposes, looked just like any other routine blood transfusion. But that half an hour of transfusion wrote a new chapter in human medicine and earned for the doctors the honour of being the world's first genetic surgeons. For the four year old girl it spelled life. During those thirty minutes of transfusion, the patient, received one billion of her own white blood cells which had been genetically altered to contain a gene which she did not have from birth.

Adenosine deaminase (ADA) deficiency is a rare inherited condition in which the person is unable to manufacture ADA, an enzyme crucial to the immune system, which provides defence to the body against infection by foreign organisms. This girl was not endowed with the gene that produces the enzyme ADA, and without this enzyme, she was extremely vulnerable to infection.

What the researchers did was to isolate the ADA-gene from elsewhere and inject it into a collection of her white blood cells. The cells so fortified were then injected into her body. The thirty minute transfusion gave her about a billion of her own white blood cells but this time with a difference. They had been genetically engineered, each incorporated with a copy of the missing ADA - gene. As such they were now armed to assist the immune system. However, there is a catch. As the life span of all blood cells is limited, she would need periodic bolstering of the genetically altered cells. A permanent cure would involve the introduction of the ADA-gene into the stem

The ADA-gene prevents the build-up of toxic metabolites

cells that give rise to blood corpuscles or her genome, such that it is forever spliced into it. In that case every new cell that is generated in the body would automatically possess the gene. It would also be passed on to her children. Such a permanently stitched on foreign gene would be a transgene. But even the periodic transfusion of the engineered cells is just a little inconvenience in comparison to the precious gift of life. And the girl's response has already helped optimistic physicians to go ahead with this form of treatment.

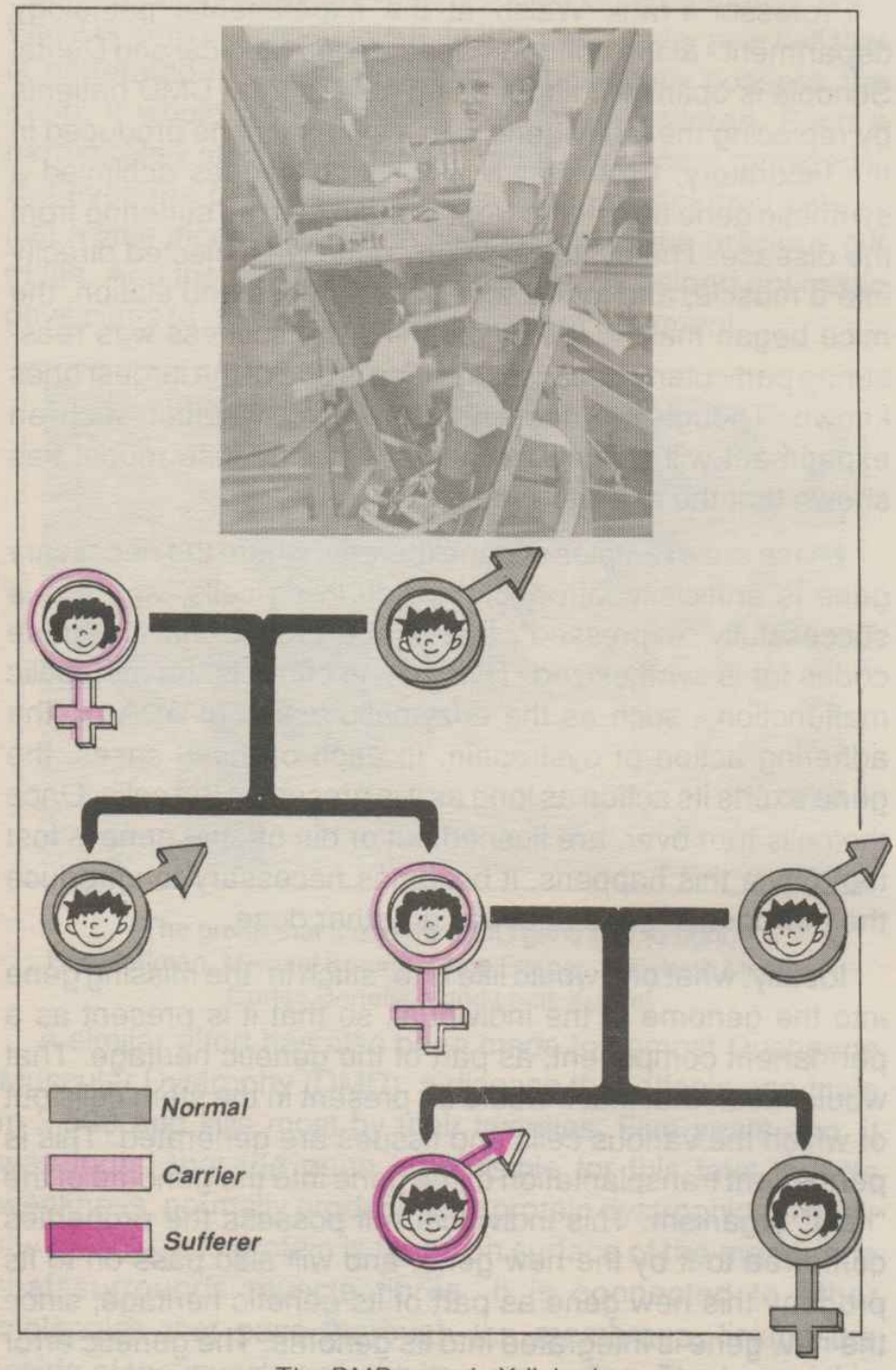
The group that found the DMD gene (left to right)
Eric Hoffman, Michael Koenig, Chris Feener, Marybeth McAfee,
Corlee Bertelson and Louis Kunkel

A similar effort has also been made to combat Duchenne Muscular Dystrophy (DMD), a disease that affects one male in 3,500 and kills most by their twenties. Five years ago, it was found that the gene responsible for this fatal muscle weakness, normally produces the protein dystrophin. In healthy muscle, this protein is found on surface of the membrane that surrounds muscle fibres. It is connected to other molecules that pass thorough the membrane, linking the inside of the muscle cell with its surrounding. The loss of this protein leads to the destruction of the muscle.

Professor Frank Walsh at the experimental pathology department - at the London-based United Medical and Dental Schools is optimistic about being able to treat DMD patients by replacing the faulty gene with a synthetic one produced in the laboratory. Prof. Walsh and his colleagues achieved a synthetic gene transfer to treat a strain of mice suffering from the disease. The dystrophin copy gene was injected directly into a muscle, and to the scientist's surprise and elation, the mice began making the dystrophin. The success was reassuring particularly because the gene is one of the largest ones known. Though it is too early to predict whether such an experiment will succeed in humans, the mouse model has shown that the strategy works.

These are examples of gene therapy, where the necessary gene is artificially introduced inside living cells, where it is successfully "expressed", that is, the protein that the gene codes for is synthesized. This protein corrects the metabolic malfunction - such as the enzymatic action of ADA or, the adhering action of dystrophin. In each of these cases, the gene exerts its action as long as it is present in the cells. Once the cells turn over, are flushed out or die off, the gene is lost too. Once this happens, it becomes necessary to introduce the therapeutic gene again as a further dose.

Ideally, what one would like is to "stitch in" the missing gene into the genome of the individual, so that it is present as a permanent component, as part of the genetic heritage. That would mean that that it would be present in the stem cells out of which the various cells and tissues are generated. This is permanent transplantation of the gene into the genome of the "host" organism. This individual will possess the properties conferred to it by the new gene, and will also pass on to its progeny this new gene as part of its genetic heritage, since the new gene is integrated into its genome. The genetic error that this individual had will then have been corrected both for itself and its progeny - a permanent solution, as it were!



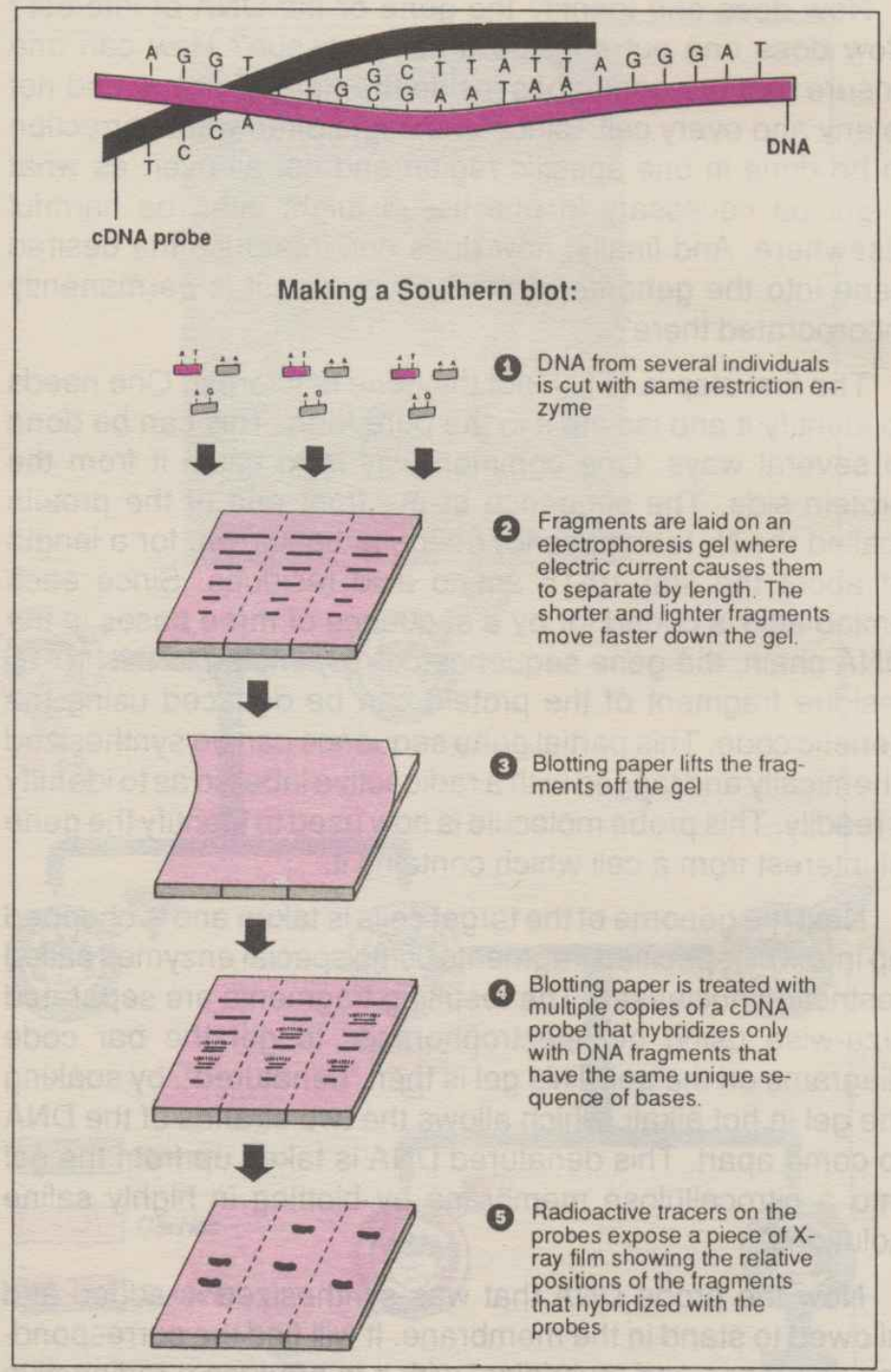

The DMD gene is X-linked.
Dystrophy afflicted limbs benefit from physiotherapy (inset)

How does one identify the gene or the DNA of interest? How does one put it inside a cell or tissue? How can one ensure that the gene goes inside the "target" tissue and not to any and every cell - since one might often want correction to be done in one specific region and not all over; as what might be necessary in one tissue might even be harmful elsewhere. And finally, how does one introduce the desired gene into the genome of the host so that it is permanently incorporated there?

The first step is to fish out the gene of interest. One needs to identify it and isolate it in the pure form. This can be done in several ways. One common way is to catch it from the protein side. The sequence of the front end of the protein (called the N- terminal end) needs to be known, for a length of about the first 10-15 amino acid residues. Since each amino acid is coded for by a sequence of three bases in the DNA chain, the gene sequence corresponding to this 10-15 residue fragment of the protein can be deduced using the genetic code. This partial gene sequence can be synthesized chemically and tagged with a radioactive label so as to identify it readily. This probe molecule is now used to identify the gene of interest from a cell which contains it.

Next the genome of the target cells is taken and is chopped up into well-specified fragments using special enzymes called restriction nucleases. The resulting fragments are separated size-wise using gel electrophoresis, to get the bar code diagrams on the gel. This gel is then "denatured", by soaking the gel in hot alkali, which allows the two strands of the DNA to come apart. This denatured DNA is taken up from the gel into a nitrocellulose membrane by blotting in highly saline solutions.

Now the probe DNA that was synthesized is added and allowed to stand in the membrane. It will find the corresponding sequence and "hybridize" with it to produce a radioactive double strand. This hybridization will be specific only with the gene of interest that we are probing with our method. The


A G G T
G C T T A T T A G G G A T
T G C G A A T A A
C A
T C
DNA
cDNA probe
Making a Southern blot:
1 DNA from several individuals is cut with same restriction enzyme
2 Fragments are laid on an electrophoresis gel where electric current causes them to separate by length. The shorter and lighter fragments move faster down the gel.
3 Blotting paper lifts the fragments off the gel
4 Blotting paper is treated with multiple copies of a cDNA probe that hybridizes only with DNA fragments that have the same unique sequence of bases.
5 Radioactive tracers on the probes expose a piece of X-ray film showing the relative positions of the fragments that hybridized with the probes

Making a Southern blot
cDNA probe hybridizing with a single strand of DNA (inset)

hybridization pattern can then be compared directly to the region of the original gel that contains the DNA sequences of interest. Now the gene of interest is identified and can be removed from the gel or membrane. This whole procedure is called Southern blotting, after Dr Ed Southern who invented this way of visualizing target DNA sequences in genes.

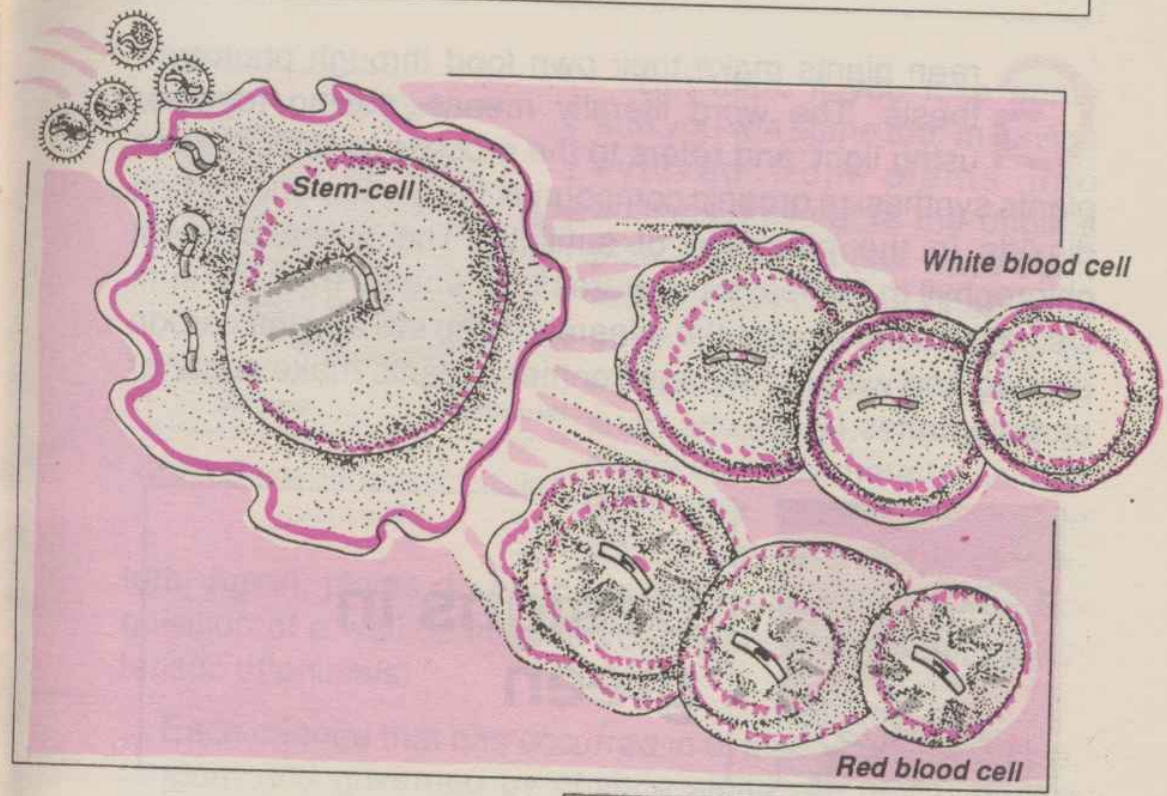
Having got the gene, how does one transfer it into the cell of interest? Several methods are possible. One can directly microinject it into the host cell using a micro manipulator - or else "electroporate" it into the cells. Here, one takes the host cells, and sends a very brief electric shock through them - a high voltage shock for a microsecond or so. This opens up pores or holes in the cell membrane through which the gene of interest can be slipped in. After the pulse of electric shock is over, the pores close up and the cell is viable again. But perhaps the easiest way is to introduce the gene of interest into a virus and let the virus infect the target cell and inject the DNA into it. This technique owes a lot to young researcher, Richard C. Mulligan who in the early 1980's devised these molecular taxis for ferrying genes into host cells. Of course, one choses innocuous or inactive viruses so as not to introduce any other infection and complications.

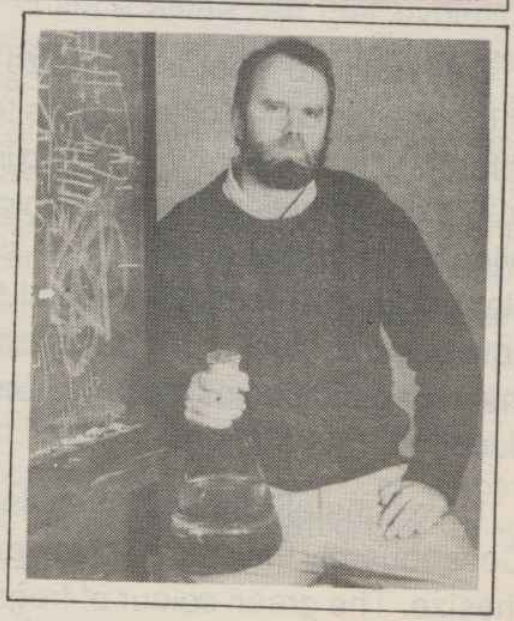
But all these techniques help in introducing only a small dose of the gene. What would be ideal is to incorporate the gene(s) in the host genome once and for all — so that every time the cells divide and proliferate, they will keep on making more of this gene and its product proteins. Such a transgenic incorporation is done by cloning the gene into the host genome.

Cloning is done using vectors or carriers that can ferry the DNA into the host cell. Plasmids are circular DNA molecules, which can be opened up and "linearized" using special enzymes. To this linearized vector DNA can be added the gene of interest and the two covalently "stitched" together using another set of enzymes called ligates, which also close up

the circle. The vector which now contains the additional gene is introduced into the host cell and allowed to multiply.

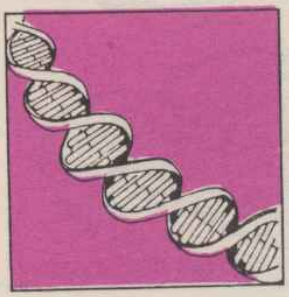
The consequences of such transgenics, and even the simpler gene therapy are remarkable. Already transgenic tomatoes, tobacco and wheat have become a reality. Last year came the success story of transgenic wheat, from the laboratory of Dr Indra K. Vasil of Florida. He isolated a specific gene that allows herbicide resistance in the wheat plant and engineered it into the wheat genome so as to make them transgenic. This transgenic wheat was cultivated in the laboratory for several generations, so as to make sure that the new gene breeds true. The seeds of this true- bred herbicide resistant wheat are now available for cultivation!



Richard Mulligan devised molecular taxis for ferrying genes into cells

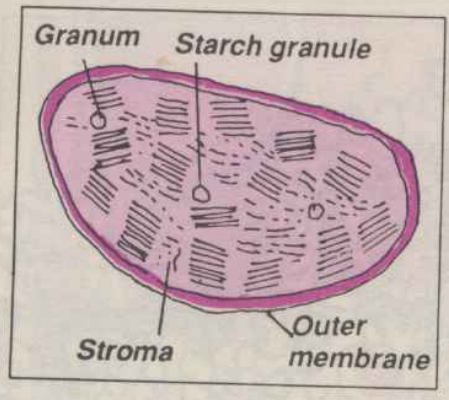
Green plants make their own food through photosynthesis. The word literally means 'putting together using light' and refers to the process by which green plants synthesize organic compounds from water and carbon dioxide in the presence of sunlight. The green pigment chlorophyll in the leaves captures light energy from the sun. Using the solar energy,the minerals of the soil and the carbon dioxide and water of the environment, plants make sugar or carbohydrates.



## Designs in green

This ability to photosynthesize rests in the small cell parts or organelles called chloroplasts that plant cells have. It is the chloroplasts that enable plants to live so cheaply and efficiently. Biologists believe that the chloroplast was actually a free living bacterium at one time that changed over to living inside plant cells in a mutually helpful fashion. Chloroplasts have now become such an indispensable part of the plants that the very definition of a plant is based on what its chloroplast does.

Unfortunately for us, animals do not have chloroplasts. Consequently they neither look green nor can they photosynthesize. The green colour of frogs incidentally does not imply the presence of chlorophyll. The habit of living cheaply has stopped with our remote ancestors that are the plants. Animals have not inherited this property of hijacking the chloroplasts and making them an integral component of their own cells. Evolution did not grant animals this advantage.

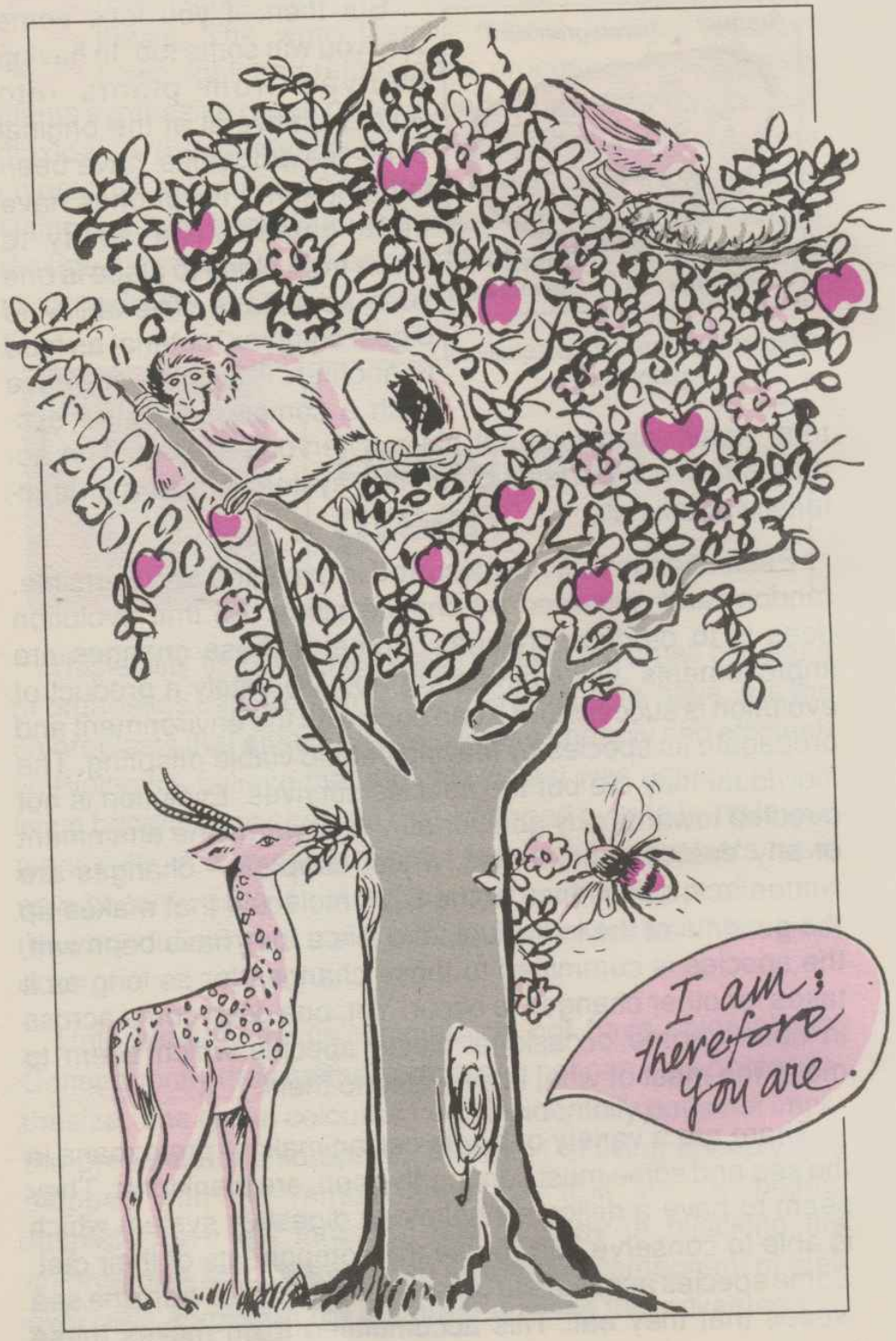


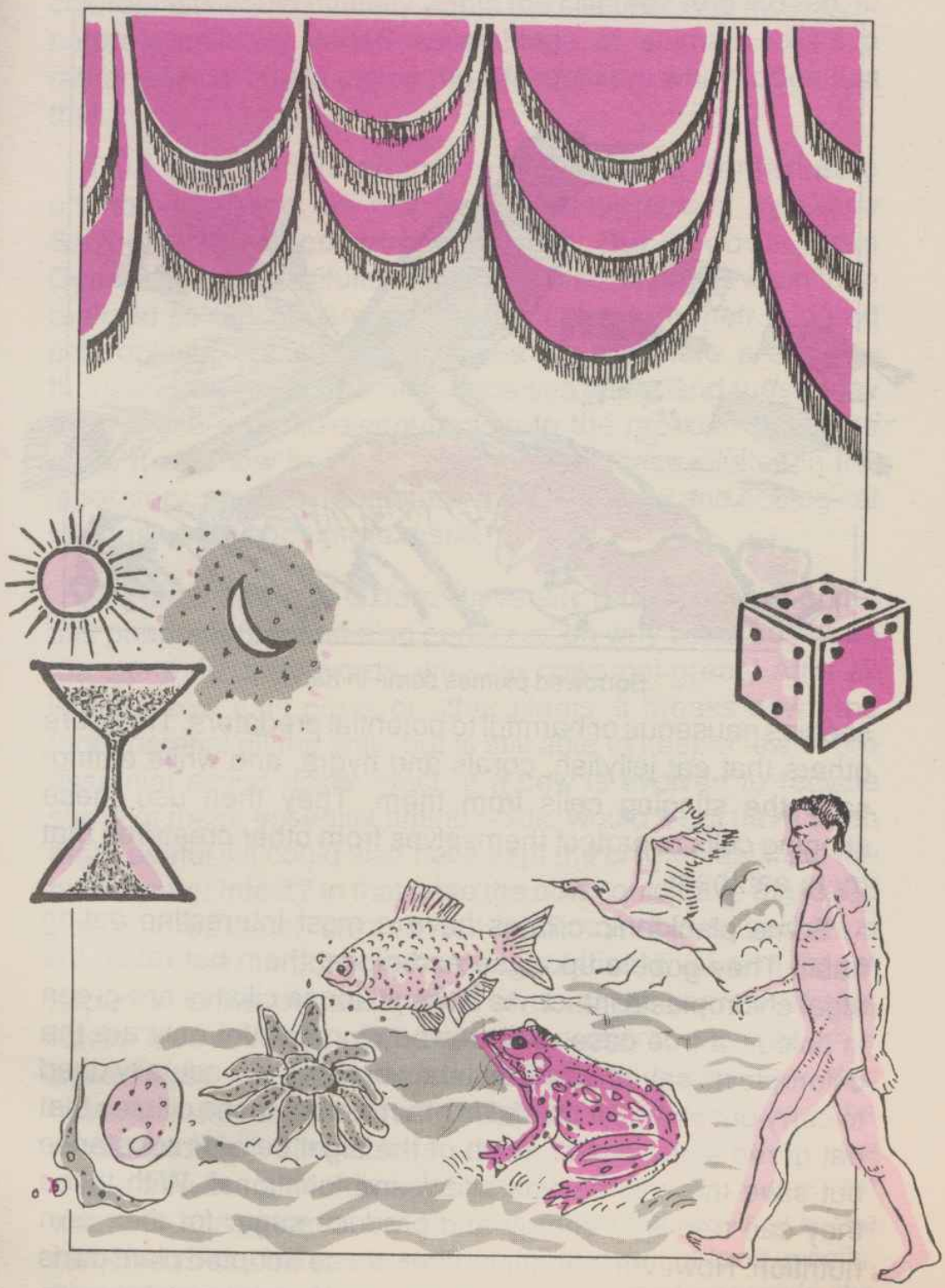

Chloroplasts are characteristic of plant cells

But then, if you lose some and you win some too. In having evolved from plants into animals, several of the original plant-like properties have been lost but some novel ones have been gained. The ability to move from place to place is one such advantage. The ability to digest complex material as food is another. Plants do not have such a complex digestive system. Again, plants do not have a nervous system. The acquisition of a well defined nervous system is also a characteristic of animals.

Each change that has occurred in evolution is irreversible, random and governed by chance alone. All that evolution does is to produce changes. Some of these changes are improvements while others are not. Ultimately a product of evolution is successful if it can cope with the environment and propagate its species by leaving behind viable offspring. The individual may die but the species survives. Evolution is not directed towards any goal, or aim, or towards the attainment of any desirable properties. It just happens - changes are written in the sequence of the DNA molecule that makes up the genome of the individual; and since they have been writ, the species is committed to those changes for as long as it takes for other changes to occur. Yet, one does come across in nature some occasional clever species which seem to make the most of what is available to them.

There are a variety of single cell animals or protozoans in the sea and some most commonly seen, are planktonic. They seem to have a delicately balanced digestive system which is able to conserve and re-use the components of their diet. Some species accumulate poisonous molecules from the sea weeds that they eat. This accumulated toxin makes these


I am; therefore you are.



Cast by chance; choreography by time

Borrowed plumes come in handy

species nauseous or harmful to potential predators. There are others that eat jellyfish, corals and hydra, and while eating, save the stinging cells from them. They then use these stinging cells to protect themselves from other creatures that try to eat them.

Some planktonic ciliates have a most interesting eating habit. They gobble up algae and digest them but retain the algal chloroplasts intact. As a result, these ciliates are green in colour, a true case of borrowed plumes! Not only are the chloroplasts saved and kept intact but they are actually used to carry out photosynthesis! In other words, these ciliates that eat green algae digest much of the algal cellular substance but save the chloroplasts intact and functional. With these they can trap light energy and produce sugar for their own nutrition. However, sooner or later, these adopted plant parts wear out and need to be replaced. Would it not be even cleverer if there could have been a way by which these

chloroplasts could multiply within the ciliates? And indeed, in nature's infinitely varied assemblage of animals exists a rather special ciliate called the *Mesodinium* which does just that.

This kind of biological recycling is a remarkable phenomenon and, as has been recently shown by Diane Stoecker and co-workers from the Woods Hole Oceanographic Institution in USA, is also more common than realized so far. Apparently, these ciliates with their adopted chloroplasts are abundant in sea water. There are a few thousands in every litre of surface sea water and these may even make a notable contribution to the productivity of the area. It has now been possible to grow these ciliates in the laboratory, so as to understand the physiology and ecological importance of such protozoans.

Dr Ralph Lewin of Oxford University has wondered about this phenomenon and also pondered on why cows do not do the same. In other words, why are cows not green! After all when a cow eats grass or other plants, it breaks down the food material all the way but is still able to keep a few of the essential amino acids intact. If a cow is evolved to recycle some of these essential amino acids, would it not have been more useful if it could also have kept the chloroplast from the plant matter intact? In that case the cow could have exploited the chloroplasts, carried out photosynthesis and thus manufactured carbohydrates for its nutrition! Evolution did not impart to cows the ability that some of the marine ciliates have. True enough, cows have been offered several other advantages - muscles, bones, nerves and brain. They also have the advantage of an adopted organelle called the mitochondrion in their bovine cells. The mitochondrion was also a free-living microbe at one time, just as the chloroplast was, but has since been taken over by other cells. It is the mitochondrion that completes the breakdown of food in cells. It uses oxygen to do so and releases a lot of energy in the process. Without the mitochrondrion, the cow would probably

have been only fermenting its food into alcohol, as yeasts do, or else turning it into curds as lactobacilli do. As to whether cows are at a disadvantage because they have not adopted the chloroplasts from the grass they eat, we do not know the answer.

In any event, cows are too opaque and too clumsy to do what the ciliates can. Too opaque because of all the fat, skin and hide that the cow needs for protection. In any case, its stomach where the grass is processed is too far away from the skin surface for light to reach. The protozoans are far thinner and hence more transparent. The cows are too clumsy because of their internal architecture. The evolutionary advantage of being able to eat grass and digest much of it, has given the cow too powerful a tool; and sifting and sequestering the chloroplasts is too delicate an operation for it to perform.

The colour green would have given the cow another advantage too. It would have helped in camouflage. Dr P.J. Stewart of Oxford University, has wondered as to how is it that while birds, reptiles and frogs are green, there are no green mammals at all. Perhaps we mammals inherited, from our drab nocturnal ancestors, a deficiency in pigmentation. In fact, many mammals are drab in their skin colour. There are no bright colours at all! At least man is able to do something about this. Brilliantly patterned and coloured dresses are perhaps a human effort towards rectifying this deficiency! But may be brighter days are ahead! Addition and incorporation of new traits at the genetic level has now become possible thanks to biotechnology. Gardeners and horticulturists have since long practised the science of grafting, selective breeding and similar techniques to introduce new traits in crops and ornamental plants. The many varieties of roses and mangoes bear testimony to their knowledge and skill. The Green Revolution has been possible with such methods of selecting advantageous generic traits. With the recent methods of genetic cloning and engineering, creation of newer forms of

Brightening up

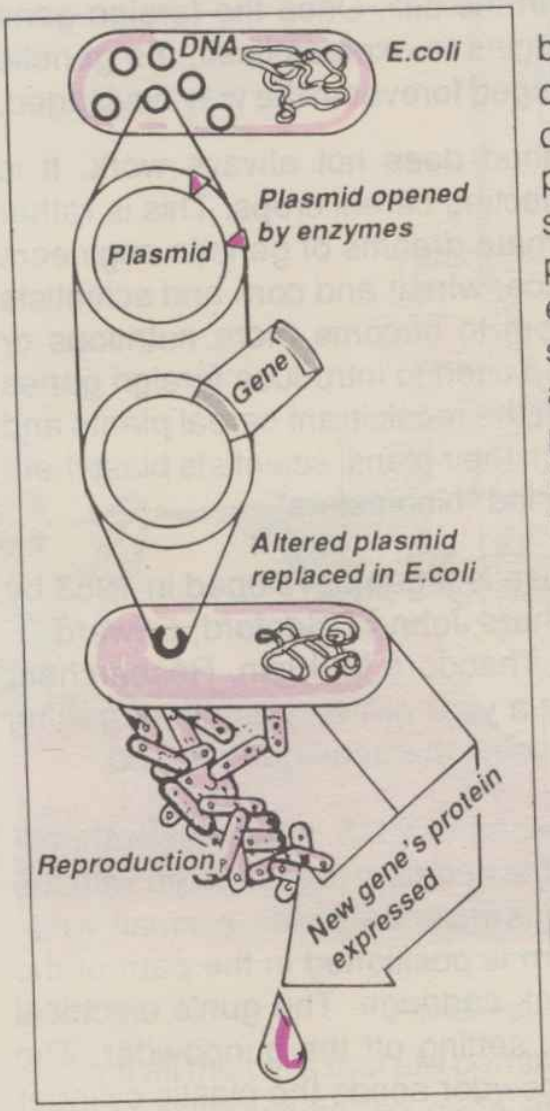
life has been possible. The techniques adopted to create "designer plants" are many and can be selected to best suit the purpose or the species under study. The introduction of foreign genes into host plants has been achieved in several instances. Chinese scientists have introduced a gene into the tobacco plant that confers to it the ability to fight and kill some invading viruses. These genetically engineered plants are thus a great economic advantage over the conventional virus-vulnerable ones.

Flowering plants are usually divided into two classes: Dicotyledeneae and Monocotyledoneae, based on whether the seed leaves are paired or single. Dicots form the larger of the two classes and include many forest and fruit trees.

Giant vegetables bear testimony to human knowledge and skill

Oaks, potatoes, beans, cabbages and roses are all dicots. Prominent among the monocots are the palm trees, bananas, grasses and cereals.

The dicots are particularly amenable to genetic engineering by virtue of the fact that they are easily infected by the bacterium, *Agrobacterium tumefaciens.* This bacterium alters the nature of the cell it enters and makes the altered cell grow unchecked, by generating tumourlike galls in the infected plants. The tumourous tissue retains the characteristics of altered cells even after the infective agent is killed by the antibiotic drug, penicillin. The bacteria had obviously left behind, or provided, a 'tumour inducing principle' in the "host" plant cell, somewhat like the Cheshire cat in Lewis Carrol's *Alice in Wonderland.* The cat disappears but the smile is left behind! But what is this tumour inducing principle?




Plasmid mediated gene transfer

It was found that many bacteria contain a circular or ringlike bit of DNA, called a plasmid. Such plasmids have given scientists one of the most powerful tools of genetic engineering. American scientists have shown that a segment of bacterial plasmid DNA integrates into the genetic material of the altered cells and thus modulates the cellular response.

While *E. coli* has been the workhorse of genetic engineers, the bacterium *A. tumefaciens* has proved to be a facile tool for the rational and deliberate alteration of plant cells. The trick was to engineer the plasmid DNA to bear the gene of choice and to exploit the bacterium's infective nature.

The bacterium carrying the doctored plasmid is used to infect surface sterilized leaves which first forms an unorganized proliferative mass of cells called callus and then shoots. The shoots are excised and rooted using special hormones and nurtured.

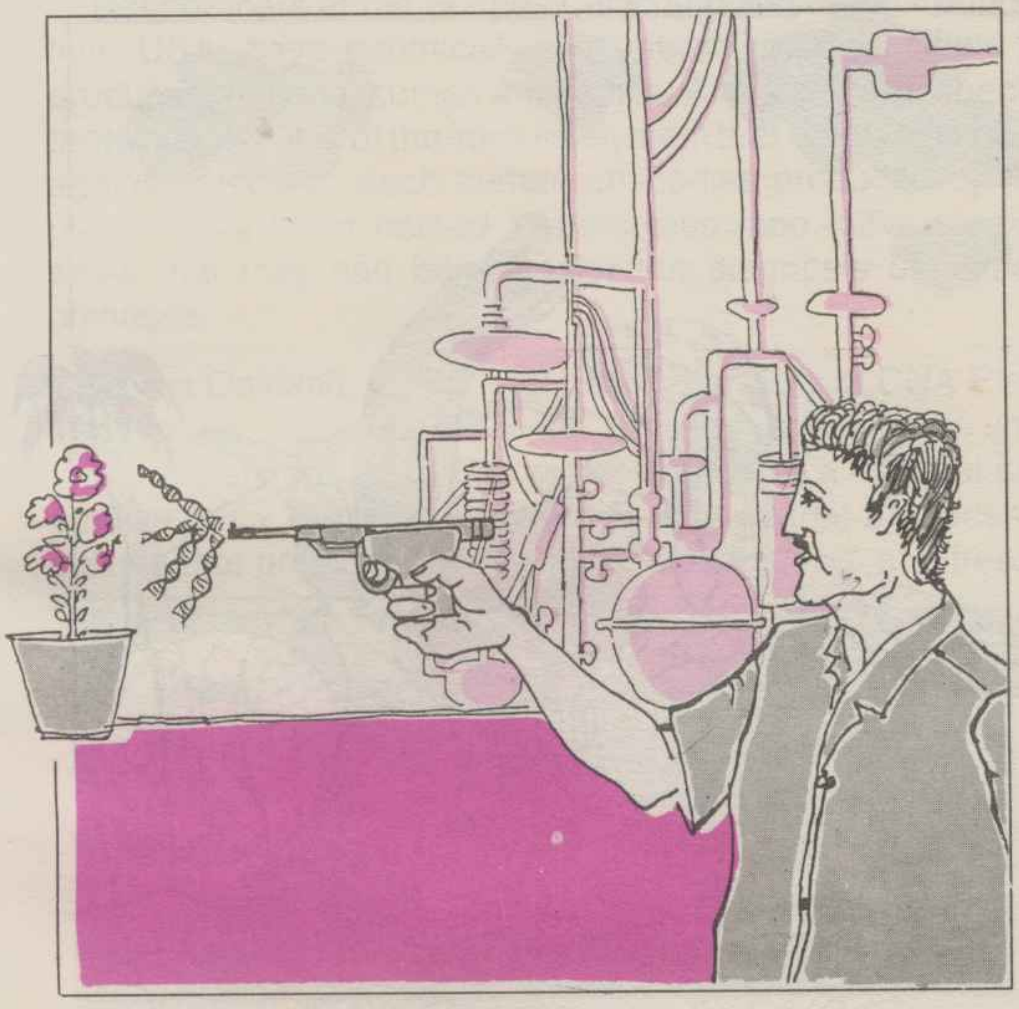
Once the plasmid integrates into the genetic makeup of the target plant it also introduces the foreign gene into the original

genetic matter contained in the cell. Once the foreign gene become operational or begins to express itself, the genetic makeup of the plant is changed forever in the way envisaged.

But the bacterium method does not always work. It is usually unsuccessful in infecting cereal crops. This is rather unfortunate, since the ultimate dreams of genetic engineers focus on staple food like rice, wheat and corn and scientists would like to engineer them to become more nutritious or more bountiful. The method used to introduce foreign genes here is more drastic. To get the recalcitrant cereal plants and other monocots to fall in with their plans, scientists blast them with special gene guns, called "bioblasters".

The bioblaster most in use is a gun developed in 1983 by Cornell University researchers John C. Sanford, Edward D. Wolf, Nelson K. Allen and Theodore M. Klein. Researchers estimate that they are only a year or two away from getting corn to accept new genes using the gene gun method.

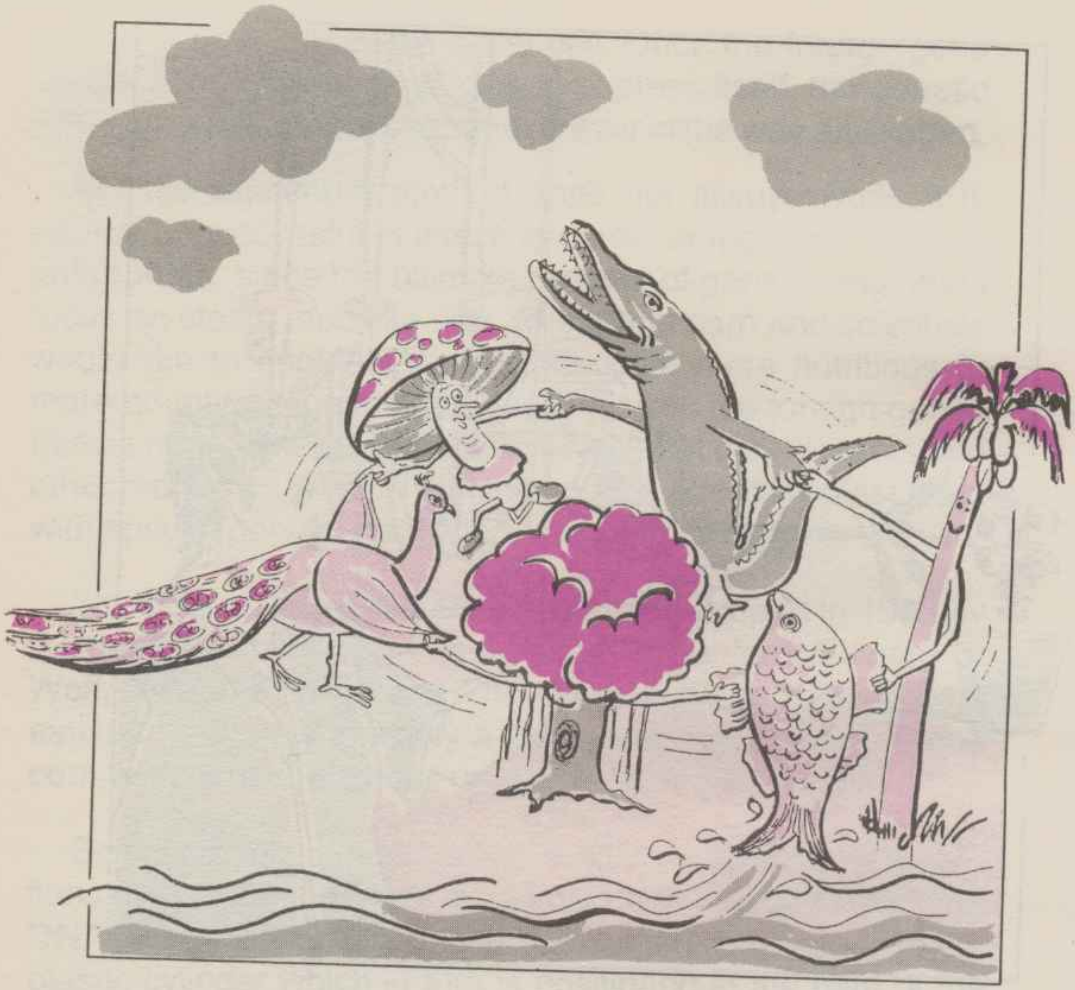
Scientists mix sample genes or DNA molecules with millions of tungsten particles, thus coating the tungsten with the DNA. This dark-grey soup is inserted inside a small white plastic cylinder which in turn is positioned in the path of the gene gun's 22- caliber blank cartridge. The gun's electrical firing pin hits the cartridge, setting off the gunpowder. The force of the exploding gunpowder sends the plastic cylinder down the gene gun's barrel and into a steel plate. The plate stops the cylinder, but its contents — the gene(s) and tungsten mixture — escape, shooting out through a small hole in the plate and crashing into the leaf, stems, embryos, or cells waiting in the petri dish below. A vacuum pump connected to the gun sucks air out of the chamber that the particles move through so that they can travel with little resistance, gaining the momentum they must have in order to punch through the tough cell wall and the cell's inner lining. The particles are so small that they need all the velocity they can get.



Hands up!

But not all the cells that are bombarded are penetrated and not every cell accepts the gene as part of its own genetic material. But the technique is extremely versatile and it has been used successfully to deliver new genes into organisms ranging from yeast to algae to higher plants as well as to animal and human cells.

A green cow might sound like the marriage between an animal and a plant. But then such differences are at the level of the organism and species. At the level of the genes, a DNA molecule is a DNA molecule, be it from plant, microbe or man. It should thus be possible to get transgenic plant-animal



Lets share genes

structures - sounds odd, but it is not really all that radical at the molecular level, is it?

Strictly speaking there should be no difference between DNA *per se* of animals and plants. In fact, scientists have succeeded in transfering genes governing the production of certain insecticidal protein(s) from the microbe *Bacillus thurengiensis* into plants such as tobacco. These transgenic or genetically engineered plants are thus made resistant to the insect pests to which they were formerly vulnerable. Plants have also been experimentally armed with toxin-producing foreign genes taken from bacteria and scorpions.

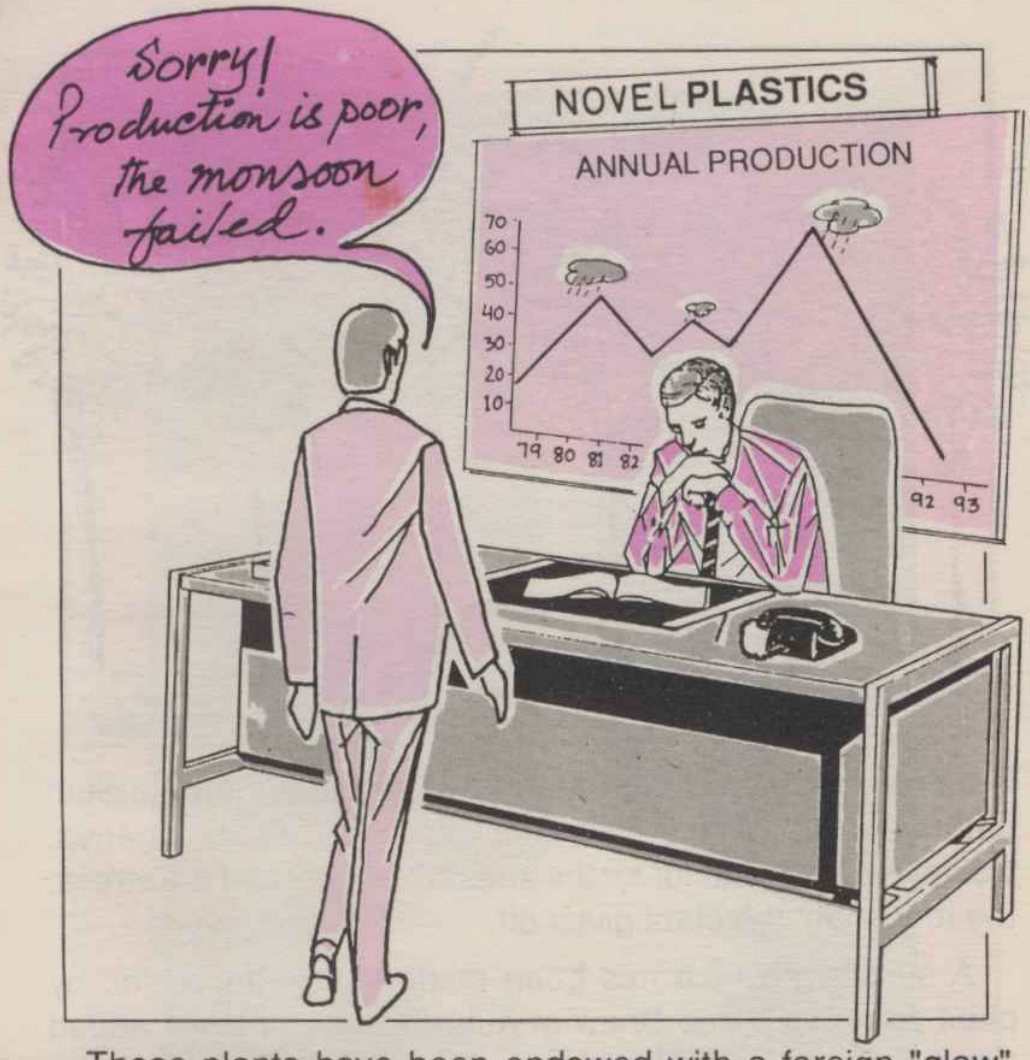
Researchers at the Scripps Clinic and Research Foundation, USA, have genetically engineered tobacco plants to produce functional human antibodies. Antibodies are special protein molecules of the immune system that defend the body against infection. Such human antibodies produced by the plants have been named 'plantibodies' and initial studies show that they can be used for the diagnosis of human diseases.

At the Oakland, California(USA), laboratory of DNA Plant Technology, researchers have inserted a fish gene into tomatoes and tobacco. The winter flounder is a fish that can survive in icy cold water, thanks to a gene that produces a protein that prevents the fish from freezing. This anti-freeze

gene has been inserted into tomato and tobacco plants to make them better resist the damages that a freeze-thaw seasonal cycle entails. Researchers have also successfully produced modified tomatoes that take twice as long to ripen than ordinary ones, and to engineer tomato, such that it would not go squashy when ripe. For a vegetable, almost a third of which rots before it reaches the market, these are remarkable achievements. Researchers at the Iowa State University plan to turn plants into efficient production factories for oil. A carrot gene has been identified which, if incorporated into plants such as soybeans, might be able to induce them to increase oil production. A rather more distant goal along this line of research is to switch plants from producing edible oils to producing high-value hydrocarbons to be used as petroleum replacements.

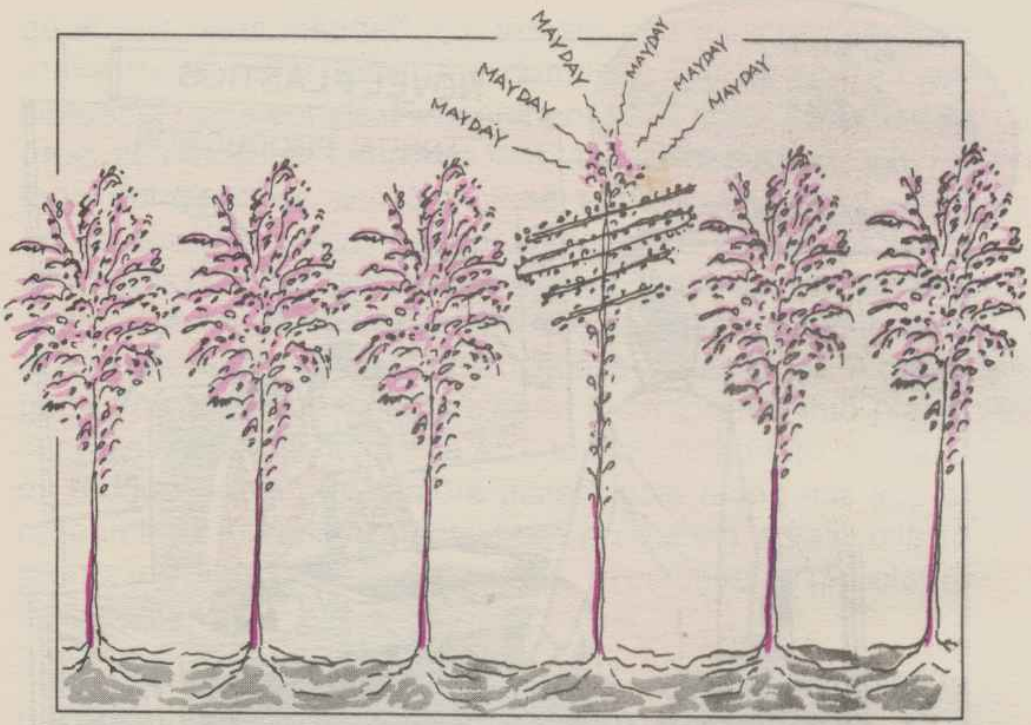
In a related field of research, scientists are contemplating the creation of plastic producing plants as a cash crop. As a first step, scientists have demonstrated that by introducing plastic producing genes into bacteria, a biological plastic "factory" is feasible. Taking this as the fore-runner, farmers may in the future grow plastics as a crop! American genetic engineers have taken the first step towards creating plants that produce plastics. They have genetically modified thale cress plants (*Arabidopsis thaliana*) to produce polyhydroxybutyrate (PHB), a natural and totally biodegradable plastic material; they believe that they can similarly target root crops like potatoes and sugarbeet.

Some other experiments of plant genetic engineering sound like science fiction! British scientists have genetically engineered plants which produce a sky-blue glow when under stress. The amount of light emitted indicates the nature of the stress. One wonders whether it might be possible to position these tell-tale indicator plants among normal crops to act as living biosensors; all that farmers might then need to assess the health of the crops would be to use hand-held light sensors.



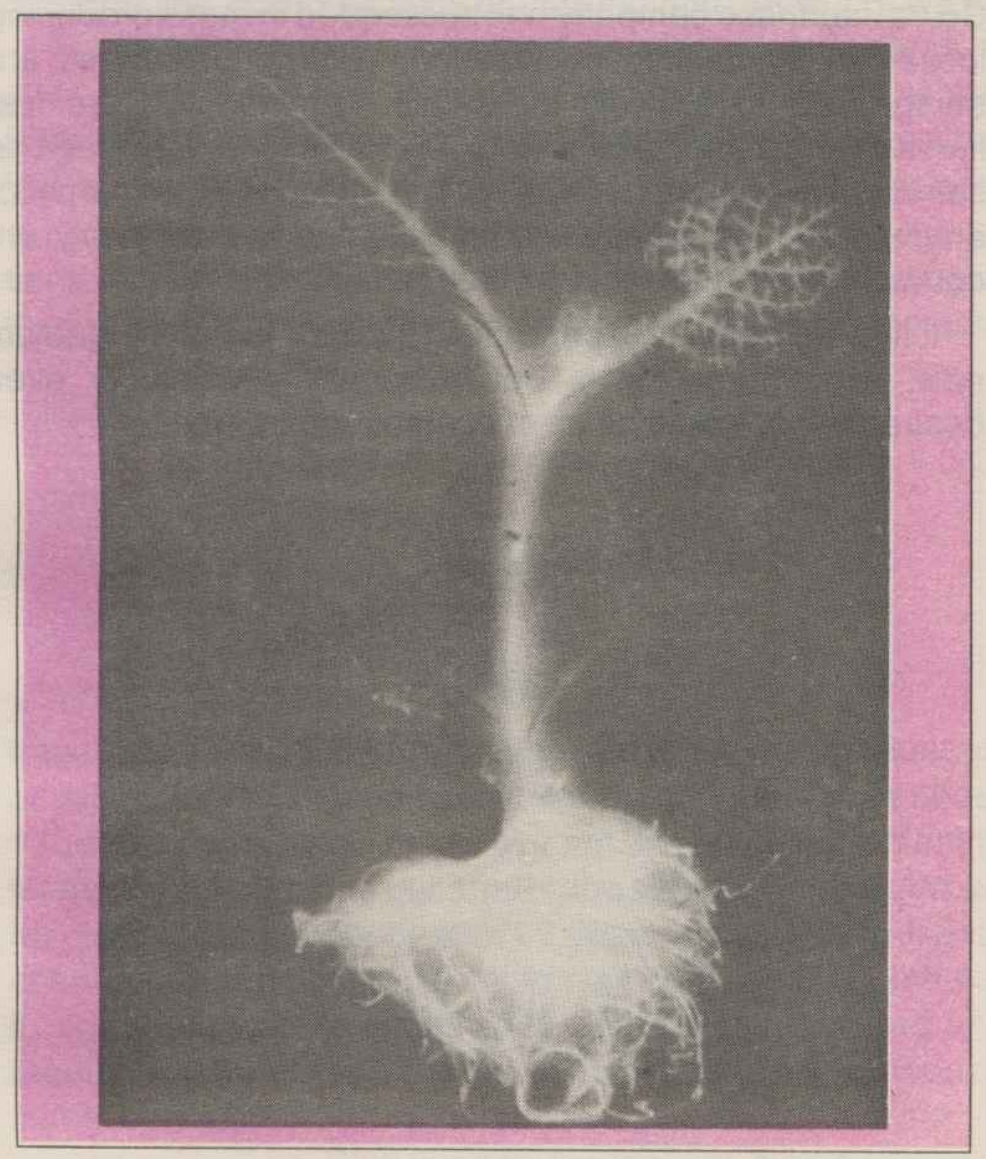

These plants have been endowed with a foreign "glow" gene which is activated when the host plant is stressed in some way. Each type of stress triggers different levels of activation, so by maintaining a record of the amount of light emitted under different circumstances, the intensity of the glow and the stimulus causing it can be correlated.

The sky-blue glow comes from a protein-called aequorin which the jellyfish *Aequorea victoria* makes naturally. This protein is sensitive to the amount of calcium ions, and emits a blue fluorescence with increasing amounts of calcium. The *Agrobacterium* plasmid was used to introduce this aequorin gene into tobacco, potato and cress as a monitor of stress. In the stressed plants, the main bodies of cells are abnormal-




ly rich in calcium. The greater the stress, the more calcium enters the cell. With the introduction of the aequorin genes, we have a light monitor for the stress. Thus greater the stress, the more light the plant gives off.

A similar advance has been made for cabbages too by plant pathologist Joe Shaw at Auburn University. He added the genes responsible for the bio-luminescence of the microbe *Vibrio fischeri* to *Xanthomonas* a deadly bacterium that causes cabbage, cauliflower and brussels sprouts to wilt. When these altered *Xanthomonas* travelled through the leaves, the path they took was clearly visible to camera. By combining a series of photographs of the same leaf into a fast-motion movie he saw the bacteria spread out into the leaf by travelling through the veins. The foreign genes that had caused the *Xanthomonas* to glow had also lit up its path which the scientists could track. Similar experiments using the "glow" genes from fireflies. are also underway. Bioluminescence and biotechnology joined hands in 1987 when the bioluminescence or *lux* genes were introduced into a virus



A glowing future when biotechnology and bioluminescence strike deep roots

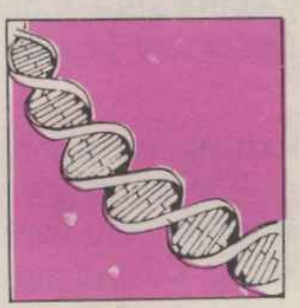
that infected bacteria. This virus served to inject the *lux* genes into the bacteria which subsequently integrated the *lux* genes into their genetic matter and glowed in the dark.

This system of making bacteria light up is tipped to be a sensitive indicator for the presence of bacteria in food. In nature, fireflies glow when they oxidize a substance called luciferin with the help of the enzyme luciferase. Kikkoman

Co., a Japanese company has succeeded in mass producing luciferase by genetic engineering. Firefly luciferases are being used extensively in assays. Luminol was the first luminescent isotope-substitute and is still widely used in commercial immunoassay kits. The success of the genetic-engineers has been literally enlightening and definitely encouraging.

It may be possible in principle to produce just any organism now, even green cows. But how easy, feasible or even desirable that would be is, of course, another matter.

The first *Homo sapiens* or thinking man, of whom we all are descendants, emerged on planet earth about a million years ago. In the long history of our planet, which is about four billion years old, this is but a flicker of the eyelids. Yet, looked at from the mortal, human timescale of decades, we have come through more than thirty thousand generations. A long path indeed, and what is even more striking is that until about 500 generations ago, we were nomadic



## Tuber tales

travellers. Hunting and gathering were our main means of obtaining food. Each nomadic tribe was no more than about 150 strong, which traveled as a group and engaged in hunting for meat and gathering fruits, nuts and plants for eating. When he discovered fire about 400000 years ago, his food habits changed for the better. He could now cook his food and eat it. Yet, there was no way that he could get an assured and regular supply of food for his daily needs. This guarantee came only about 500 generations ago, when mankind invented agriculture. With this new technology, man could sow, grow and harvest rice, wheat and other cereals for staple food, and "squirrel" away the harvest in a granary for future use.

Agriculture revolutionized the human way of life in many ways. It transformed his life from the nomadic to the pastoral. It made settlement into village communities possible and organization of societies on a larger scale became the hallmark of man. It freed him from "thinking on his feet" all the time and afforded him some leisure time for other pursuits - such as art

and culture. It also led him to conquer and colonize territories, and spread himself in more efficient ways.

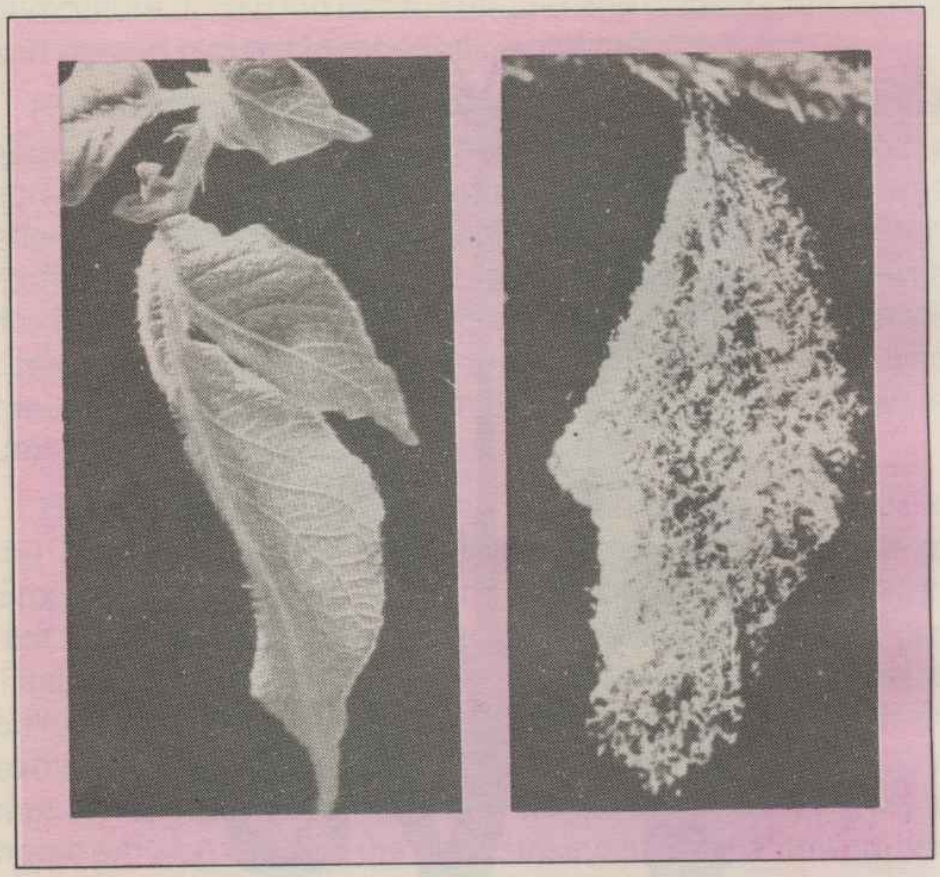
Human biologists estimate that agriculture in Europe started in the Near East around 10,000 BC and steadily spread westward at the rate of a kilometre a year. This was effected both by people moving and settling into new territories - conquering them by force or peacefully mingling with the local people. Such 'demic diffusion' brought new practices, new methods and new technology to the area. It also brought in new plants and animals to regions where they were unknown before.

The rose plant is one such. The Turkish Ambassador to the Moghul Court in Delhi brought the rose as a gift to Empress Noor Jehan. She was so enchanted by the flower that she arranged for its propagation in various parts of India. That was hardly 400 years ago — and today who would believe that this exquisite flower is not native to India but an exotic import! Moving from the ornamental to the more mundane, many vegetables that we eat in our daily meals at home are also imports, the most striking of which is the potato.

Wild potatoes are found from Nebraska (USA) to Chile but plant explorers have reported the greatest diversity among potatoes growing in the Andean region of Peru, Ecuador, Bolivia and Argentina where potatoes were cultivated perhaps 2,000 years ago. But what does Indian agricultural practice and its history tell us? In *The Hindu's* letters to the Editor column of October 21, 1991, S.Ramabhadran of Udhagamandalam recalls how potato cultivation was the main occupation in the Nilgiris until 30 years ago and how the potato blight disease wiped out the produce from the 35,000 acres in 1964. Expert suggestions on using blight-resistant strains, pesticides and fertilizers were not heeded as these were too expensive. Instead, farmers took to tea plantation in the Nilgiris because there was a feeling that potato might not be considered a staple food in India, where cereals, pulses and grams have been popular from time immemorial.

The potato in 1589. A brilliant future ahead

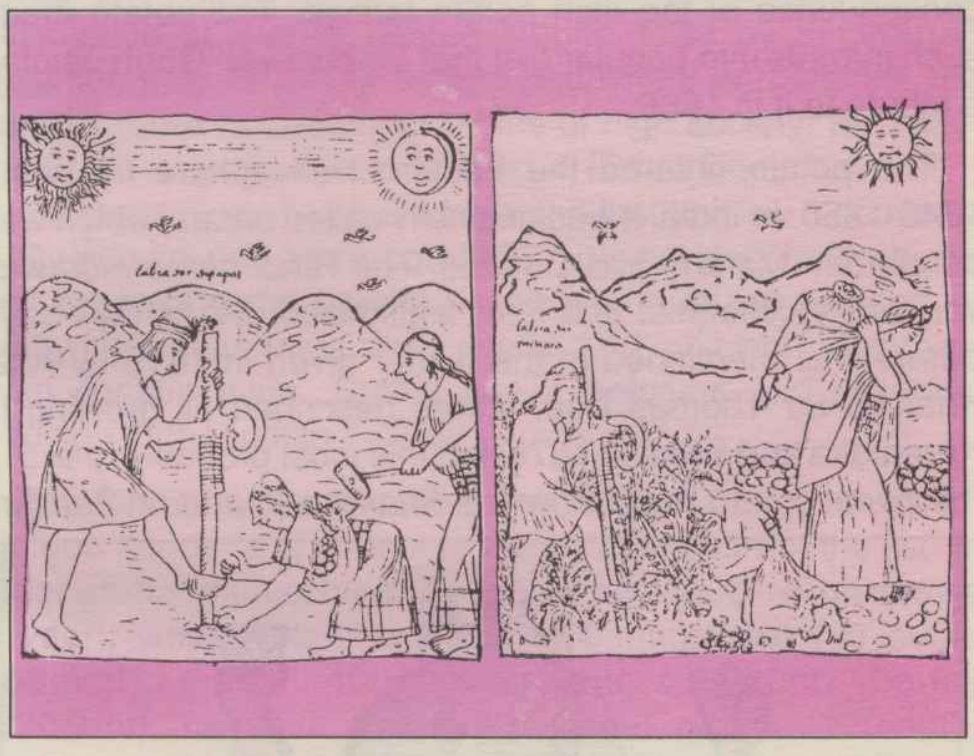
This belief triggers the question about the antiquity of the potato in India. In his forthcoming book, "*Indian Food a History*" the famous food scientist Dr K.T. Achaya of Bangalore dwells on this point. While the *Encyclopaedia Brittanica* places the potato, as a native of the Peruvian- Bolivian Andes, cultivated over conservatively speaking, 1,800 years ago. Dr Achaya feels that it was cultivated as early as 5000-2000 BC. Its common name is derived from the word *batata* from the Taino language of the American-Indians and the extinct aborigines of the Greater Antilles and Bahamas, and especially Haiti; but it actually refers to the sweet potato. The Spaniards brought the tuber from Southern American to Europe around 1570 and *Batata* became the modern potato.



Blight can wipe out potato harvests

Dr Achaya says that the real Spanish name, seldom used, is *Papa*, used for the potato in New York City during the Sixties.

Within a century of entering Europe, the potato became the major crop of Ireland and by the late 18th century in Europe. Ireland by then had become totally dependent on potato cropping. Thus when late blight struck during 1845-1860, one third of the Irish starved to death and others succumbed to typhoid that followed. The great potato famine resulted in many Irish moving to the UK and other parts of Europe and to the New World of the United States. They took with them their language, customs and wit, as also the Catholic church. Many citizens of the New England States of northeastern America trace their history to this demographic

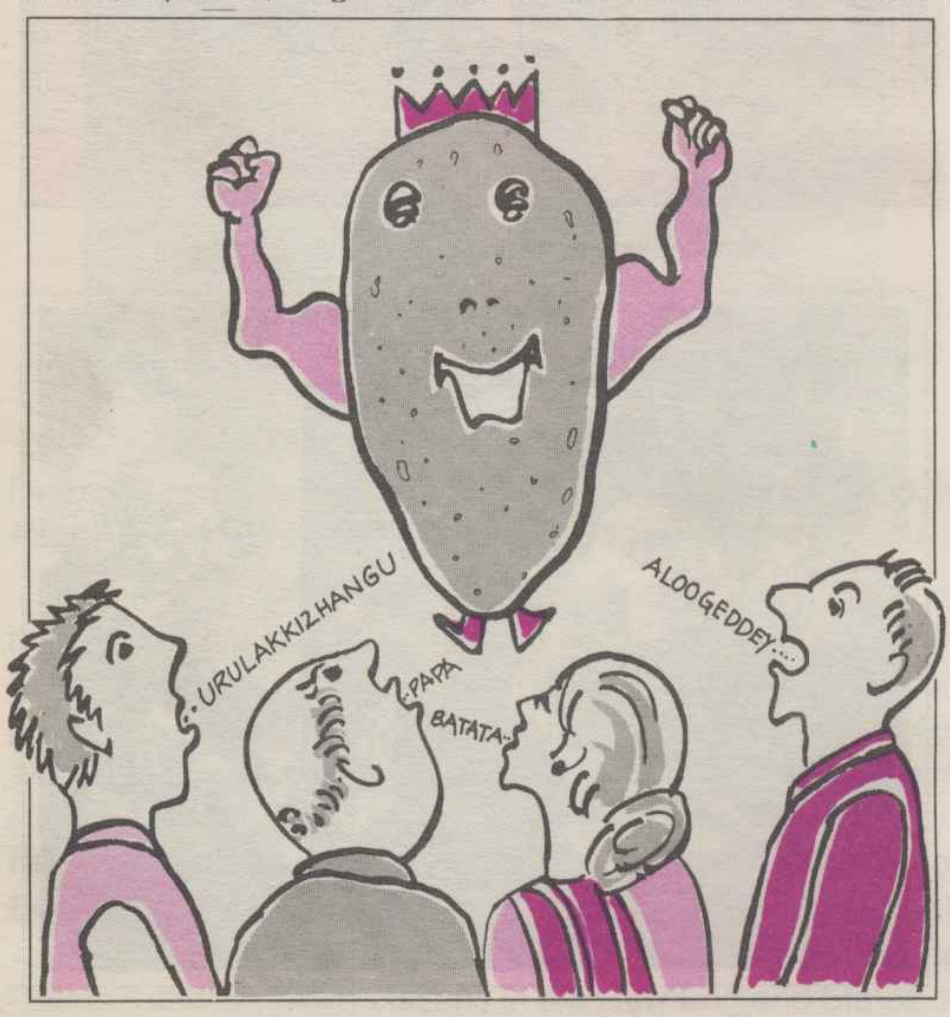


Planting and harvesting potatoes in Inca times

'The Potato Eaters' by Vincent van Gogh

consequence of the Irish potato famine. The potato made such inroads into popular diet that Vincent van Gogh painted a tribute to it in 1885.

The potato entered the Eastern Hemisphere by about 1840-1850. In India, it was originally called *batata*, which was adopted in Marathi and Gujarati. The Hindi name *aloo* was very likely colloquial and later legitimized. Dr Achaya says *batata* was mentioned in the dinner given in 1615 by Asaf Khan to Sir Thomas Roe. It was described as growing in Karnataka and Surat in 1675; but this most probably refers to the sweet potato, long known as *Sharkara khanda* in Sanskrit



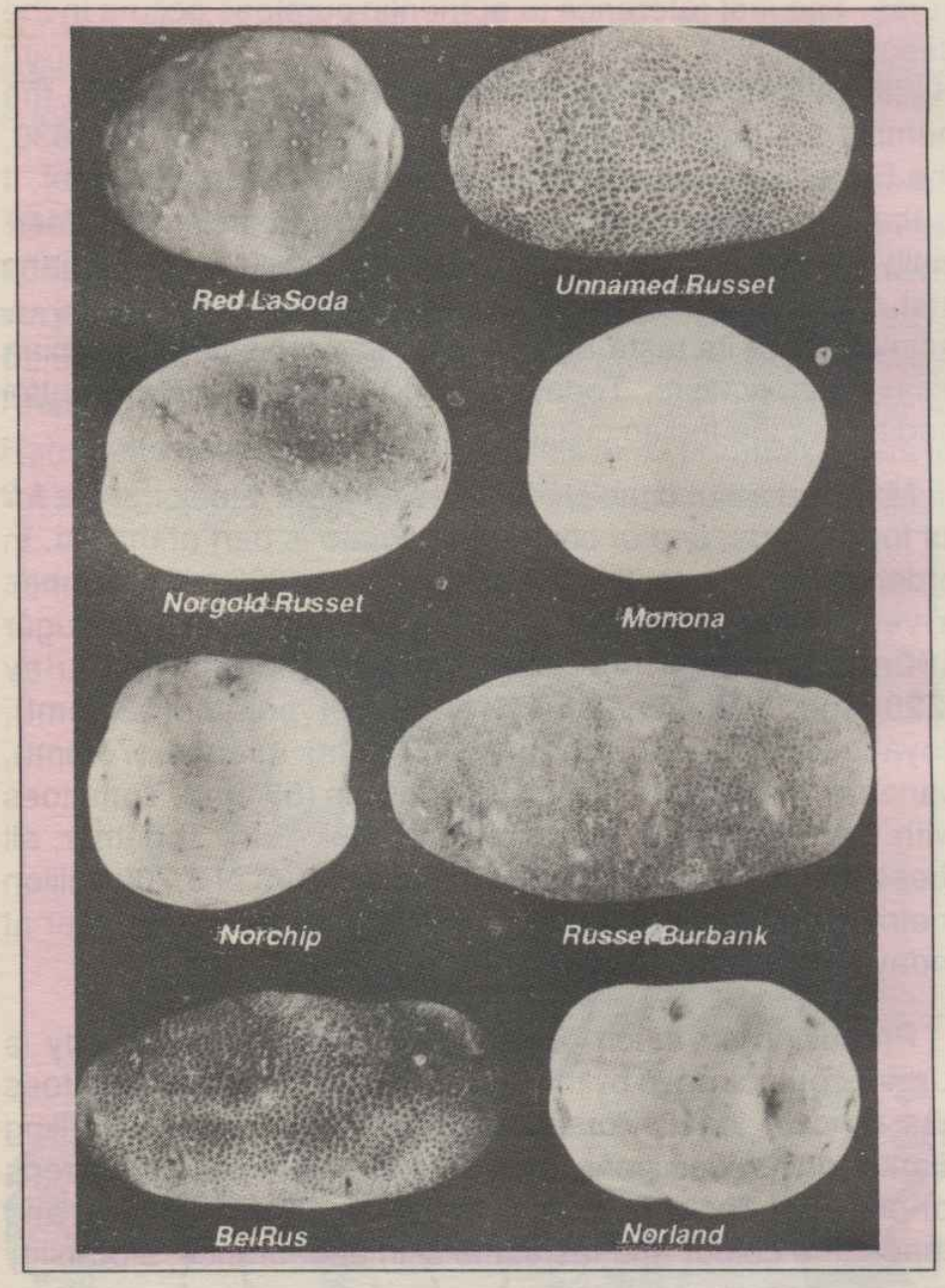

The '*sahasranama*' of *Solanum tuberosum*

verse. The first reference to authentic potatoes occurs in the description of a meal hosted around 1780 by Sir Warren Hastings; this no doubt helped popularize and elevate the humble tuber to the dinner tables of high society. By 1830, the British took up potato farming in Dehra Dun. However, it is the Dutch who may have been the first to grow it commercially in India, perhaps around 1800. It is not clear how Indians first reacted to the potato; possibly with mixed feelings — enjoyment of its taste and novelty, and inhibition stemming from conservatism. Today, it is a welcome and delightful addition to the Indian cuisine.

Mankind around the globe relies on just 14 major crops for its food needs, and of course the potato is part of the list. In order of importance, these are sugarcane (800 million tonnes a year), wheat (500mmt), rice (400mmt), beet (for sugar 400mmt), maize (400mmt), potato (300mmt), barley (220mmt), cassava (150mmt), sweet potato (120mmt), soyabean (100mmt), sorghum (80mmt), grapes (70mmt), bananas and plantain (60mmt), and oats (55mmt). Tomatoes with their 50 million tonnes a year, rank 15th. Together, all these foodcrops constitute a total production of 3,700 million metric tonnes, or about half a tonne per person each year at today's population rate.

Producing so much potato is not easy. The difficulty is further compounded by the fact that each market for potatoes has its own ideal. Potato producers face several baffling market-driven decisions each time they purchase the seeds for planting. Their choice has largely to do with the size and shape, the colour and taste, the skin appearance, storability and processing properties.

Growing potatoes was a chancy endeavour until the mid-nineteenth century but today, biotechnologists can virtually give farmers a designer potato of their choice. They could have superb baking or chipping potatoes meant to be baked or turned into french fries and chips before being eaten.




Potatoes come in many shapes, sizes and colours

A potato has to be high in solids or dry matter in order to be considered worthy of making good chips. Such a potato does not soak up as much oil during frying as does a lower-solids potato; this is a bonus for both the chips manufacturer

*Hum ko* chips *mangta*!

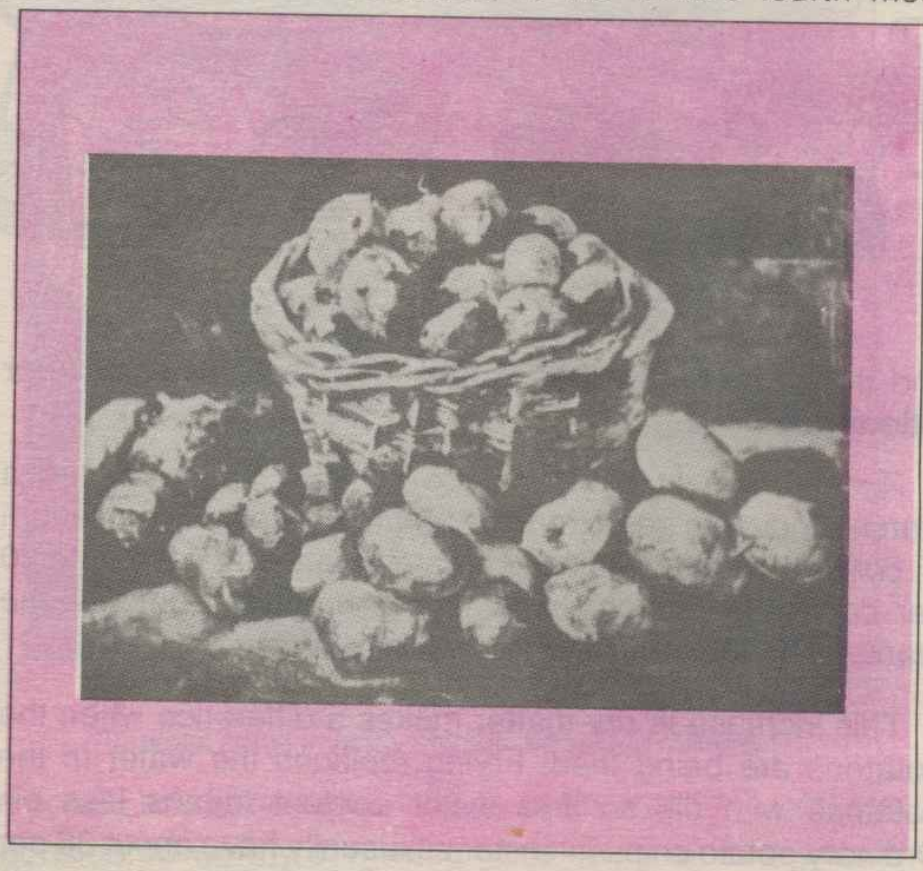
and the user, since oil is expensive as well as calorific or fattening.

Researchers at Monsanto Co. in USA have inserted a starch producing gene from the common intestinal bacterium *E. coli* into potatoes so as to create tubers that have upto 20 per cent more starch than the best potatoes now on the market.

This increase in dry matter makes a difference when the potatoes are being fried. Frying replaces the water in the potatoes with oil, so less water content means less oil. Ordinary potato chips for instance, usually have about 36 per cent oil but the new improved version would contain only about 30 per cent. The six per cent decrease in oil content

may sound small, but consumer awareness and economic criteria could well tip the scales in its favour.

Meanwhile parallel advances in genetic engineering have yielded super-potatoes. Calgene-Pacific, a Melbourne based biotechnology company has announced that it has isolated a gene that is capable of producing twice the normal number of tubers. This new kind of potato, expectedly termed 'superpotato' will yield double the amount per hectare against the conventional kind. Calgene Pacific claims that the innovation will help the Third World by slashing production costs. They also say that the potato provides more nutrition from less land and in less time than rice, wheat or corn. The potato grown in about 130 countries, is the world's fourth most



May the harvest be rich
Still life by Vincent van Gogh

important food and an increase in its yield could have considerable impact on world food production.

Field trials are now on and the superpotato will also have to be tested for its taste when fried, mashed or baked. If it fails these tests, it might meet the same fate as the 'pomato', a hybrid between the potato and tomato, developed in Florida a decade ago. But scientists are more eager to test its vulnerability to diseases and pests, for the potato as a species, is prone to a host of them.

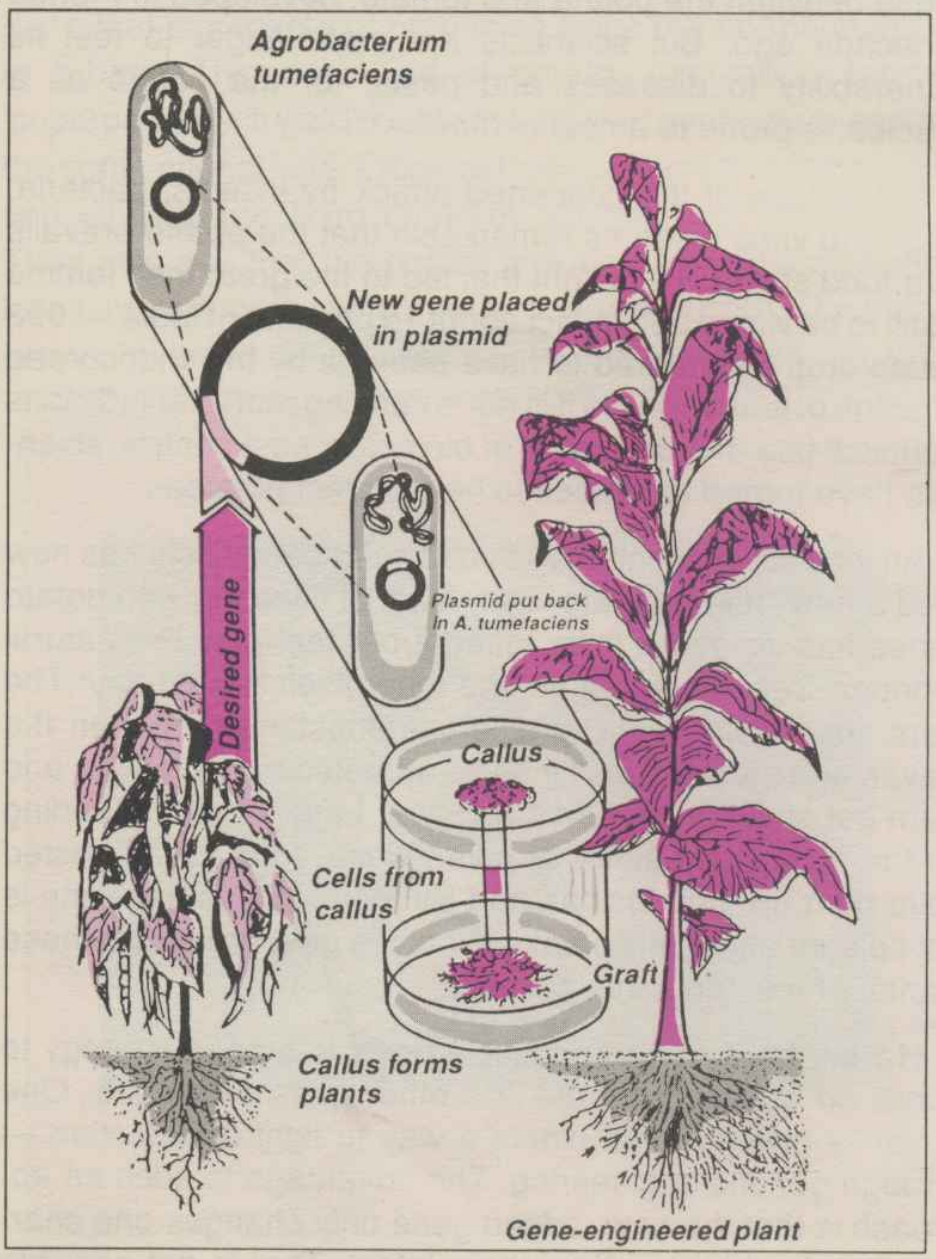
In the face of the concerted attack by insects, bacteria, fungi and virus it seems remarkable that the potato prevails as a food staple. The blight that led to the great Irish famine is still to be wiped out. In fact about 15 per cent of India's 1992 potato crop was feared to have been hit by the widespread infection of leaf blight, so it is not surprising that after judicious chemical use and programs of biological pest control, scientists have turned to biotech to help protect potatoes.

An international collaboration of plant geneticists has now bred a new "hairy" potato. Ten years of tinkering with potato genes has yielded a tasty tufted tuber that acts like natural flypaper. Despite the name, the tuber itself is not hairy! The hairs tipped with a sticky glue like substance grow on the leaves and stems. Small insects attracted to the leaves and stem get stuck and die of starvation. Larger insects feeding on the hairy parts suffer an even worse fate. The ingested hairs gum up their insides and kill them. (Of course, one is not so sure about this potato. What if a goat grazes on these plants, or man eats the leaves?)

Having hairs that trap insects pests is a good strategy to adopt no doubt, but there are other possibilities too. One option is giving potato plants a way to fight off infection — through genetic engineering. The advantage to such an approach is that the new, added gene only changes one characteristic and leaves the others intact. That is not possible with conventional breeding because every cross results in

new and different offspring that may be close to, but not exactly the same, as the parent.

Scientists can add new genes to many broadleaf plants like potatoes, tomatoes, and tobacco with help from the commonly used vector *Agrobacterium tumefasciens*. The




Engineering better potatoes

bacterium contains a plasmid, a tiny piece of DNA, that enters a plant's chromosomes during the infection process. Because the DNA actually becomes integrated into the plant's DNA it is a perfect carrier for introducing foreign genes into plants. *A. tumefasciens* itself has been genetically altered so it does not cause the typical infection signs.

Potatoes of the future may boast new genes that enable them to fend off attack by disease, withstand harvest-time bruising, or go untouched by the chemicals that kill adjacent weeds. Because biotechnology allows scientists to borrow genes from other organisms so the new potatoes may even boast of genes from a moth or perhaps from a chicken egg! Experimental modification using these genes is already under way.

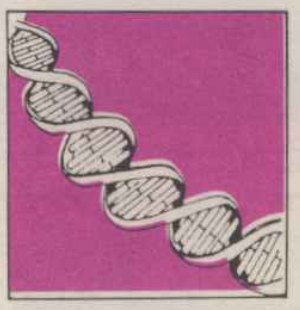
Potato plants were genetically modified two years ago at the ARS Process Biotechnology Research Unit at California, USA. For one test Dr William R. Belknap modified a gene taken from the greater wax moth, a grayish brown pest. The moth's gene may protect potatoes from blackspot bruising — harvest-time damage that blackens potatoes beneath their skin.

How could a moth's gene help a potato? The enzyme that makes the black color of a potato's bruise uses an amino acid called tyrosine. The greater wax moth has a protein that stores large amounts of tyrosine. The protein is the product of a moth gene. When inserted into a potato, the gene might change tyrosine's location and form within tuber cells. That might make tyrosine less accessible to the blackening enzyme.

Perhaps the most intriguing of the genes targeted for experiments is the one cloned from chicken eggs. An antibacterial gene, it protects the vulnerable embryos until the chicks develop their own immune system. Researchers are hoping the gene will similarly defend potatoes from two major diseases caused by bacteria — soft rot and ring rot.

The time, energy and resources being devoted to the culture of *Solanum tuberosum* has surely indicated that growing potatoes is no small potato of a job. Particularly notable in this context is the suggestion made by Dr John Niedeuhanser of Tucson, Arizona, USA, the 1990 winner of the World Food Prize, and who is called "Mr Potato". He suggests that if potato is cultivated on a far larger scale than it is now, it can help stave off hunger in future, when the population of the world doubles and resources do not. He advocates turning the potato from a side dish into a staple food. Citing history as example, he avers that the high productivity of potatoes might have fuelled the industrial revolution of the 18th and 19th centuries!

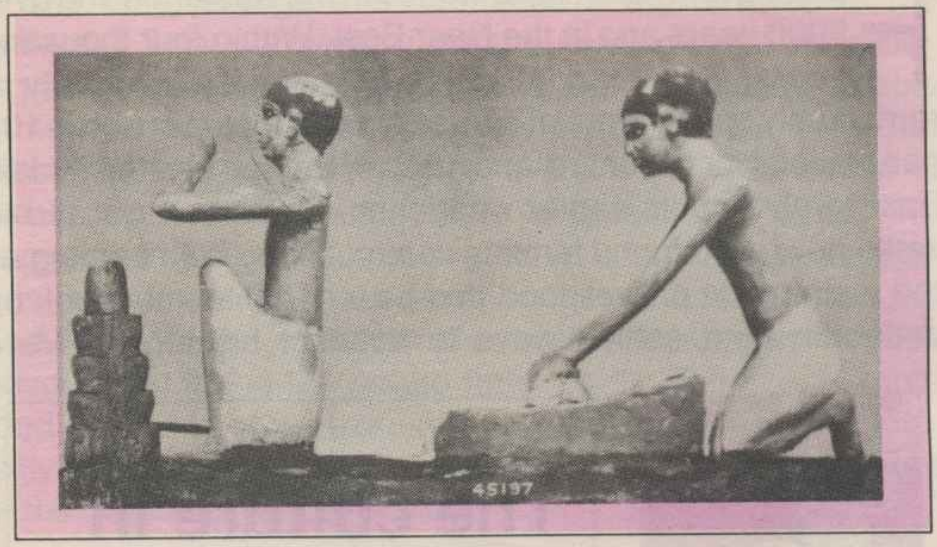
Farming and agriculture started in the western world over 9000 years ago in the Near East. Within four thousand years, it spread all over Europe. This is truly a remarkably speedy rate of spread since it took just about 100 generations for the entire land mass of Europe and the Middle East to give up its earlier wandering nomadic ways and to settle down and found farming communities. With farming as the main mode of livelihood and pattern of life, man took on sedentary and settled ways to conduct his affairs. More



# The culture in agriculture

leisure and security became available than what was possible in the nomadic times. Technology began developing and advancing. Living off a knapsack does not permit the development of elaborate technology; it can at best be pack- and -move, or carry-on technology. Nothing that is of a permanent nature was permissible. That required a more settled community, since a sedentary and serene milieu kindles and promotes advances in technology more efficiently.

The mathematician Jacob Bronowski remarks in his great book "The Ascent of Man" that what is surprising at first glance is that it took man so long in history to take to farming. Given that *Homo sapiens* or thinking man emerged on earth close to a million years ago, why was agriculture 'invented' only as late as 9,000 years ago? A likely reason for this delay is that it was only about 10,000 years ago that the last Ice Age ended. With that, the earth began blooming and was ready for farming. Before then man had to move around as a nomad, but with the Ice Age lifting, settling down appeared a possible and desirable option.



Let there be bread for all.

Ancient bread making depicted on an Egyptian tomb of about 2000BC △
An Indian farmer at the wheat mill ▷

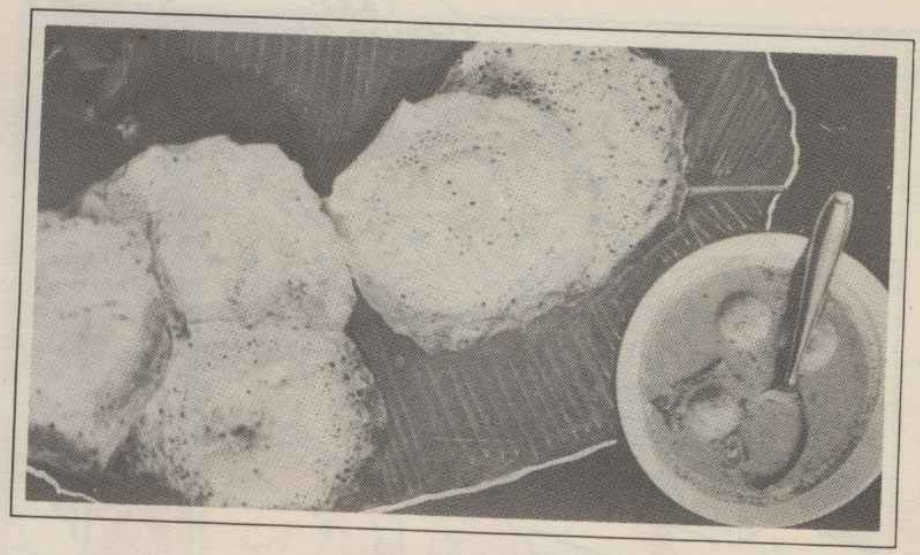
Even more interesting is Bronowski's observation on what turned out to be a crucial mutation in the wheat plant that seems to have occurred around ten thousand years ago. Prior to that, the common variety of wheat was the emmer, which is a spikelet containing two grains and thus a foraging crop. It propagated in the wild, through broadcasting or the scattering of the seeds in the wind. Around 10,000 years ago, the emmer crossed with another grass resulting in the present day bread wheat. This cross produced a beautiful ear of wheat, which was fundamentally different from the ear of emmer. The new one had a tight ear that would not break easily, and had to be broken by someone else. This wheat is

well packaged, can be stored and cultivated to a plan. Man was thus offered a plant that he could use and exploit to advantage. Wheat, on its part, was waiting for man to help it propagate - a true case of "made for each other" or symbiotic coexistence.

An aspect of interest is the pattern and mode of spread of agriculture. That it spread westward from Mesopotamia has been established by following the appearance of artefacts such as jars, implements and the like all over Europe. The question is – how did it spread? Generally speaking, there are two modes by which such spreading occurs. One is what may be termed as cultural diffusion. Here, the methods, the concepts and the preliminaries are learnt and passed on. The idea is imported from the source, absorbed and modified to suit local conditions. Music offers several excellent examples of such cultural diffusion. Some ragas of Hindustani music, such as Zilaf Kafi, can be originally traced to Persia and the Arab region. Music directors of Hindi or Tamil movies who revel in plagiarizing and lifting tunes from Europe or America are agents who aid in such cultural diffusion. For those who might snigger at the use of the adjective cultural in this context, a more acceptable and profound example is the spread of science and the method of science.

In contrast to the cultural diffusion is what may be called the migrationist mode. This simply means that people move from one place to another in numbers, and when they do so they take their habits, culture and technology along. In more technical terms, this is referred to as demic diffusion. The term demic is derived from the Greek word *Demos* for people — and is seen in words such as endemic (restricted to a group of people), epidemic (across a population) and the like. Cuisine is an excellent example of the manifestation of demic diffusion. *Idli*, *Dosai* and *Vadai* are household names in Northern India, thanks to the migration and settlement of people from the south. In this instance, the mere export of the idea is not enough. It needs the substantial and daily

Artefacts — the chroniclers of agriculture



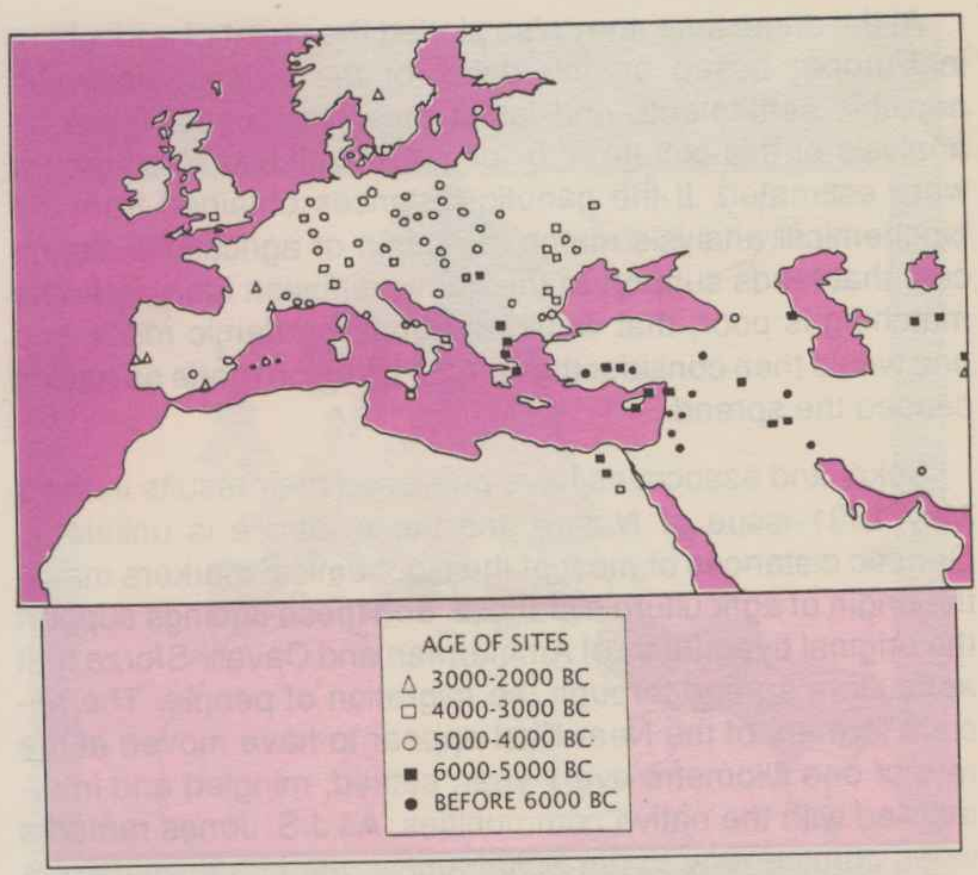
Traditional fare

presence of South-Indians in order that the *Idli* be authentic; otherwise it becomes a cultural variant of the dish. This is the complaint of the Italians about the dish pizza which is popular in America; though it is originally an Italian dish, it has metamorphosed over the years into an uniquely American entity - very different from the pizza of Italy.

What about the spread of agriculture all over Europe? How did it spread? How does one decide which mode is operative-cultural or demic diffusion? As can be expected, debates rage in scholarly circles on this issue. The crucial difference between the two has been highlighted by Drs Ammerman and Cavalli-Sforza, and it is simple. It may be summarized in the telling phrase recently used by Dr J.S. Jones of University College London, which is: "when people move, they take their genes with them". If agriculture spread by demic diffusion from the Middle East to Europe, one should be able to detect genetic lineages, or phylogenic connectivities between modern day Europeans and the people of the Near East. In contrast, had agriculture spread culturally or through oral and written means, the only remnants left in the sands of time would be earthernware, implements, manuscripts and so on.

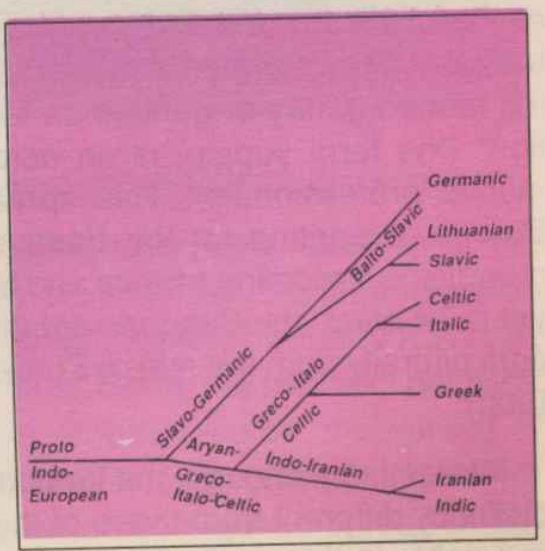
While cultural diffusion leaves artefacts behind, demic diffusion bequeaths biology to posterity.

Dr Robert Sokal of the State University of New York at Stony Brook and associates have exploited this unique property of demic diffusion in an effort to decide on the question of the spread of agriculture in Europe. They decided to look at the genetic variations and similarities in 26 different proteins and enzymes present in the bodies of people from as many as 3373 different sites spread all over Europe. The similarities help in tracing the genetic ancestry, while the differences aid in estimating what may be called the "genetic distance" between groups of individuals. This is somewhat like drawing up a family tree. The similarity and identity lead one to draw up the main lineage or the trunk of the tree, and the differences trace the branching out of cousins from the




△ Diffusion of Agriculture

Tree model of the origins of the Indo-European languages ▷

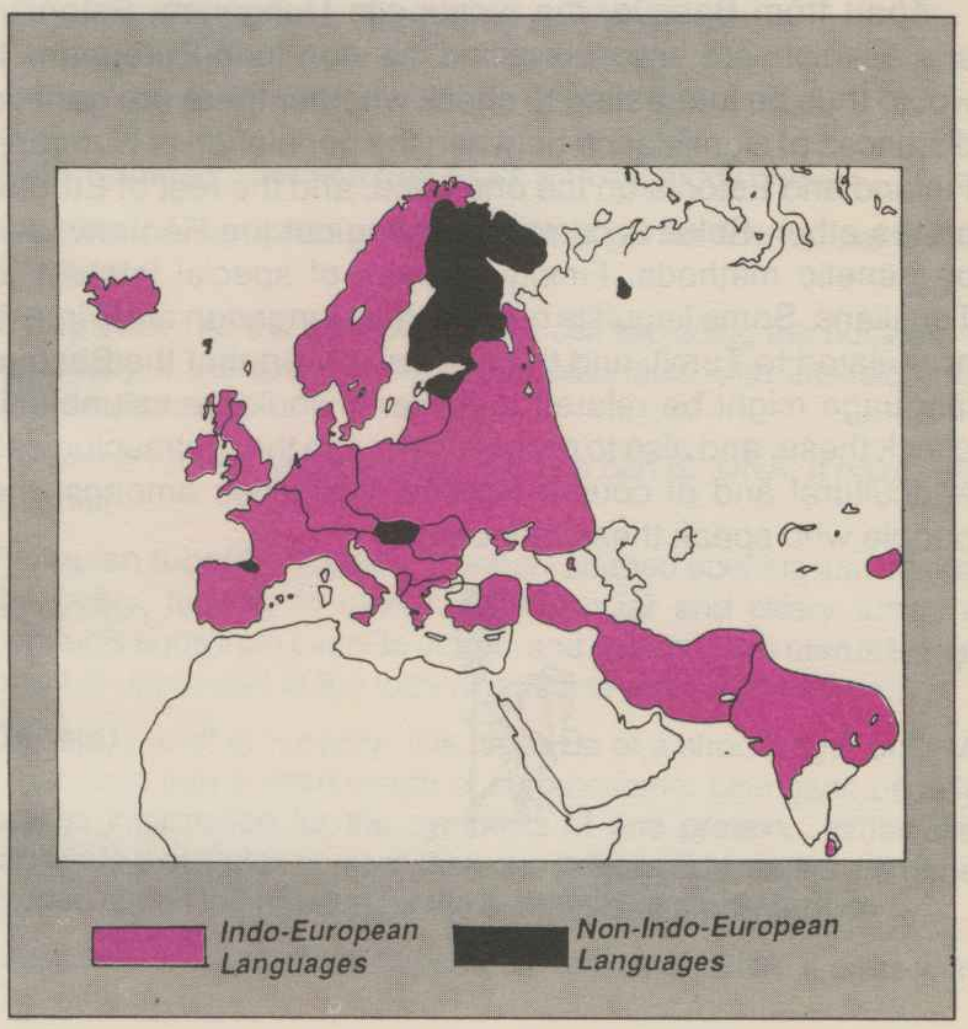



trunk line. The 26 genetic marker systems that Sokal and associates used ranged from blood proteins, **immunoglobulins** and enzymes.

At the same time, they also plotted the onset of agriculture in Europe, based on the dates of the earliest observed neolithic settlements and latest mesolithic ones. From an analysis of this picture, the "origin-of-agriculture" distances were estimated. If the genetic distances obtained from the biochemical analysis match the origin of agriculture distances, that lends support to the demic diffusion idea. If thethe matching is poor, that would discount the demic mode and one would then consider the cultural diffusion mode as having caused the spread.

Sokal and associates have published their results in the 9 May 1991 issue of *Nature* and the evidence is unilateral. Genetic distances of most of the biochemical markers match the origin of agriculture distances, and these findings support the original hypothesis of Ammerman and Cavalli-Sforza that agriculture spread through the migration of people. The ancient farmers of the Near East appear to have moved at the rate of one kilometre every year, settled, mingled and intermarried with the native communities. As J.S. Jones remarks in his commentary to the Sokal article, the hunter-gatherers of Mesolithic Europe underwent a sociological change in the bargain. They suffered a process of "gentrification" (becoming landed gentry or gentlemen farmers) or "even yuppification" (the term yuppie, is an acronym for young upwardly mobile professionals)! This spread appears true all over Europe, excepting for the Basque region of the Pyrenees mountains straddling France and Spain. For reasons that are yet to be clear, the Basques are distant both genetically and agriculturally from the rest of Europe (and the original Middle East).

Interestingly enough, the language of the Basques is also distinctly different from those of the Indo-European family. It is notable in this connection to hear the views of Professor Colin Renfrew of Cambridge University. Renfrew is an archaeologist who has studied the archaelogical pointers to language spreading, and a very readable account of his ideas




Indo-European languages are distributed from Ireland to India

on the origin of Indo-European languages appears in the October 1989 issue of *Scientific American*. It is his contention that the Indo-European languages spread throughout Europe not by conquest and imposition, but by the peaceful diffusion of people and of agriculture. In other words, the spread of language in Europe has not been through cultural diffusion or conquest (as English, French or Portuguese spread in the colonies), but through demic diffusion.

In essence, Renfrew equates early Indo-Europeans with early farmers.

Apart from Basque, the languages Hungarian, Estonian and Finnish are also classified as non-Indo-European. It would thus be interesting to check whether there are genetic distances of significance between the population of Hungary, Finland and Estonia on the one hand, and the rest of Europe on the other. Sokal is currently testing out the Renfrew idea by genetic methods. Finally, a point of special interest to Tamilians. Some linguists believe that Hungarian and Finnish are related to Tamil, and there is one report that the Basque language might be related to it too. It would be valuable to check these, and also to probe further into the archaeological, agricultural and of course genetic similarities amongst the people who speak these languages.

# GLOSSARY

**Anthropology** : Science that deals with the study of man.

**Adenine** : A nitrogenous base which is part of DNA. It pairs with Thymine.

**Cytoplasm** : All the protoplasm of a cell excluding the nucleus. It is usually a transparent, slightly viscous fluid with inclusions of various sizes.

**Cytosine** : A nitrogenous base which is part of DNA. It pairs with Guanine.

**Fallopian tube(s)** : Tube with funnel - shaped opening just beside the ovary, leading to uterus. By muscular and ciliary action it conducts eggs from ovary to uterus, and sperms from uterus to that place in upper part of the tube where they fertilize the ovum.

**Gene(s)** : Unit of heredity. It is made up of a short length of DNA which is in turn a short length of chromosome. One gene usually carries information for the synthesis of one protein. When the protein is a complex of more than one polypeptide chains the gene carries coded information for the synthesis of a polypeptide

**Guanine** : A nitrogenous base which is part of DNA. It pairs with Cytosine.

**Hormone** : Organic substance produced in minute quantity by ductless glands and transported by blood to parts distant from the site(s) of synthesis to target organs where it excites activity. The word hormone comes from the Greek word 'hormaein' meaning 'to excite'. Hormones, unlike catalysts are used up during the process of activation.

**Immunoglobulins (Ig)** : Special globular protein molecules synthesized by the body in response to infections by disease causing organisms or foreign bodies called antigens. Immunoglobulins are also called antibodies. There are five types of such antibodies, namely, IgG, IgA, IgM, IgD and IgE.

**Isotopes** : Atoms of the same element that differ in atomic mass because they have different numbers of neutrons. The number of

protons remains the some. Radioactive isotopes are used in medicine for research, diagnosis and treatment of disease.

**Mutations** : Spontaneous or induced change in the base sequence of DNA. The most important important mutations are those occurring in the ova or sperms since they can produce inherited changes.

**Paleontology** : Study of organic life in the past based on the observation of fossils and fossil imprints.

**Radioactive** : Having the property of radioactivity, which is the process of spontaneous disintegration of unstable atomic nuclei accompanied by the simultaneous release of energy in the form of ionizing radiation.

**Sequence of bases** : The order in which the nitrogenous bases are arranged in a DNA molecule.

**Sequence specific enzymes** : A special class of enzymes that cleave DNA at specific sites. Most recognize a sequence of six nuclelotides, but some five or four.

**Thymine** : A nitrogenous base which is part of DNA. It pairs with Adenine.

**X-ray diffraction** : A technique used to determine the characteristic feature of material structure. The technique involves determination of the pattern of scattering of X-rays when they interact with the atoms of a crystal. It is also used for the determination of the structure of biological molecules.

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## About the Author

A Bhatnagar Award Winner and a Fellow of the Indian National Science Academy, **Dr D Balasubramanian** (b. 1939) is one of India's leading lights in life sciences research. After doing his M.Sc. from BITS, Pilani, and Ph.D. from Columbia University, USA, Dr Balasubramanian taught at IIT, Kanpur. He is a visiting professor at the Jawaharlal Nehru Centre for Advanced Scientific Research, Bangalore. Originally a physical chemist, he gradually switched over to molecular biology via biophysics. He is at present the Director of Centre for Cellular and Molecular Biology, Hyderabad.

Apart from authoring a large number of research papers in various scientific journals and text books of school and college levels, Dr Balasubramnian is a popular science writer *par excellence*. His articles have appeared in many popular newspapers and magazines including *The Hindu, Newstime* and *Kishor Bharati. Genes and Means* is his first popular science book.

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